

Empria longicornis species group: taxonomic revision with notes on phylogeny and ecology (Hymenoptera, Tenthredinidae)

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Abstract

The *Empria longicornis* species group (Hymenoptera, Tenthredinidae) is revised. *Empria japonica* Heidema & Prous, **sp. nov.** is described from Japan. The lectotypes of *Empria gussakovskii* Dovnar-Zapolskij, 1929, *E. konowi* Dovnar-Zapolskij, 1929, *Poecilosoma longicornis* Thomson, 1871, *P. mongolica* Konow, 1895, and *P. tridens* Konow, 1896 are designated. *Empria konowi* Dovnar-Zapolskij, 1929 (**syn. nov.**) and *E. gussakovskii* Dovnar-Zapolskij, 1929 (**syn. nov.**) are synonymized with *E. tridens* (Konow, 1896), and *Empria waldstaetterense* Liston, 1980 (**syn. nov.**) with *E. alector* Benson, 1938. *Empria alpina* Benson, 1938 and *E. minuta* Lindqvist, 1968, earlier misidentified as *E. gussakovskii*, are treated as valid species. Nine species are confirmed to belong in the *longicornis*-group: *E. alector*, *E. alpina*, *E. basalis*, *E. japonica*, *E. loktini*, *E. longicornis*, *E. minuta*, *E. mongolica*, and *E. tridens*. Distributional data of the species and a key to the imagines are provided. *Ex ovo* rearings of the four most common and often misidentified species in the group (*E. alector*, *E. basalis*, *E. longicornis*, and *E. tridens*) were carried out to verify their host plants and male conspecifics. External morphology, morphometrics (geometric and traditional), and DNA sequences (mitochondrial COI and nuclear ITS1 and ITS2) are used to delimit species within the group and to assess their phylogenetic relationships. Mitochondrial DNA sequences analyzed are mostly regarded as unsuitable for species circumscription, DNA barcoding and for reconstructing species phylogeny within the group. In this regard, analyses of the ITS sequences yielded more concordant results.

Key words: Sawflies, nomenclature, taxonomy, new species, new synonymy, lectotype, mitonuclear discordance, DNA barcoding, cytochrome c oxidase I, internal transcribed spacer

Introduction

The sawfly genus *Empria* belongs to the subfamily Allantinae within Tenthredinidae. Currently with 50 valid species-level taxa (Taeger *et al.* 2010) *Empria* is one of the largest genera among Allantinae. Species identification within the genus is difficult and members of some species groups can hardly be separated even by the structure of genitalia. One of the most taxonomically problematic in the Palaearctic region is the *Empria longicornis* species group (Benson 1938; Lindqvist 1968). Until the present study six valid nominal species have been included in this species group: *E. longicornis* (Thomson, 1871), *E. tridens* (Konow, 1896), *E. konowi* Dovnar-Zapolskij, 1929, *E. alector* Benson, 1938, *E. basalis* Lindqvist, 1968, and *E. loktini* Ermolenko, 1971. *Empria basalis*, *E. konowi*, and *E. loktini*, are poorly known taxa reported only from few locations (Table 1). Three other species in the group (*E. alector*, *E. longicornis*, and *E. tridens*) are more widespread in the region (Zhelochovtsev & Zinovjev 1988, 1996; Taeger *et al.* 2006). Morphological evidence would also allow *Empria gussakovskii* Dovnar-Zapolskij, 1929 to be included in this group (Zhelochovtsev & Zinovjev 1988).

TABLE 1. Previously reported distribution and host plants of *Empria longicornis* species group. The cited distributional records are probably only partially correct because of possible misidentifications. Question mark (?) indicates the host associations made without *ex ovo* rearings.

Species	Reported distribution	Host plants
<i>E. longicornis</i> (Thomson, 1871)	Palaearctic (Zhelochovtsev & Zinovjev 1988, 1996; Taeger <i>et al.</i> 2006)	<i>Rubus idaeus</i> , <i>Filipendula ulmaria</i> (?), <i>Fragaria vesca</i> (?) (Kontuniemi 1951; Zhelochovtsev & Zinovjev 1988)
<i>E. tridens</i> (Konow, 1896)	Palaearctic (Zhelochovtsev & Zinovjev 1988, 1996; Taeger <i>et al.</i> 2006)	<i>R. idaeus</i> (Lorenz & Kraus 1957; Miles 1936), <i>Geum</i> (?) (Conde 1934)
<i>E. alector</i> Benson, 1938	Palaearctic (Zhelochovtsev & Zinovjev 1988, 1996; Taeger <i>et al.</i> 2006)	<i>F. ulmaria</i> (Kontuniemi, 1955)
<i>E. basalis</i> Lindqvist, 1968	Estonia, Finland, Sweden and Russia (Lindqvist 1968; Viitasaari <i>et al.</i> 1998)	<i>Geum rivale</i> (this study)
<i>E. loktini</i> Ermolenko, 1971	Sakhalin island, Russia (Ermolenko 1971)	(?) (1)
<i>E. konowi</i> Dovnar-Zapolskij, 1929	Volgograd oblast and Stavropol Krai, Russia (Dovnar-Zapolskij 1929)	(?)
<i>E. gussakovskii gussakovskii</i> Dovnar-Zapolskij, 1929	Finland, Ukraine, and Russia (Lindqvist 1968; Zhelochovtsev & Zinovjev 1988, 1996; Zombori & Ermolenko 1999) (2)	(?)
<i>E. g. alpina</i> Benson, 1938	Holarctic (Lacourt 1999; Smith 1979; Taeger <i>et al.</i> 2006)	<i>Dryas octopetala</i> (V. Vikberg, personal communication)

(1), the adults of *E. loktini* have been collected from *Filipendula kamtschatica* (see Ermolenko 1971).

(2), the record from Russia is that of the *E. gussakovskii* lectotype (*E. gussakovskii* is regarded here as a synonym of *E. tridens*).

The *longicornis*-group is defined morphologically on the basis of penis valves, characters which readily separate it from other *Empria* species. However, penis valves are highly similar in most species within the species group. This structural similarity has led to taxonomic inconsistencies. For instance, Hellén (1940) treated *E. tridens* (Konow, 1896) as a variety of *E. longicornis* (Thomson, 1871), and tentatively lumped also *E. alector* Benson, 1938, and even *E. alpina* Benson, 1938 together with *E. longicornis*. Conde (1940) also suggested that *E. tridens* might be only a variety of *E. longicornis*. Two subspecies of *Empria gussakovskii* (Table 1) are sometimes regarded as separate species (Lacourt 1999). Because the species can be assigned to the *longicornis*-group with confidence only by studying the male genitalia, additional members of this species group could likely be found among species the males of which have still remained unknown.

Despite the doubts expressed by Hellén (1940) and Conde (1940), the species within *E. longicornis* group can be identified consistently by studying their ovipositor structure (Lindqvist 1968; Zhelochovtsev & Zinovjev 1988); however, reliable identification of their males using present keys (e.g. Zhelochovtsev & Zinovjev 1988) is impossible.

Since no discrete qualitative differences between the males of the different *longicornis*-group species have been found, application of traditional as well as geometric morphometrics for species differentiation has to be considered. In traditional morphometrics statistical methods are typically applied to sets of measured distances, or simple ratios can be calculated from the data. Geometric morphometrics is based on landmark coordinates (two- or three-dimensional) of homologous anatomical loci, which are subjected to multivariate statistical analysis after differences in overall size, position and orientation of the specimen are eliminated. This allows comparison of differences in geometric shape of different objects. A major advantage of geometric morphometrics compared to traditional morphometrics is that measurement of all possible distances between selected landmarks is unnecessary. Geometric morphometrics is being increasingly applied in taxonomic studies (e.g. Mutanen & Pretorius 2007; Rufino *et al.* 2006; Villemant *et al.* 2007; for a review see e.g. Mitteroecker & Gunz 2009). Although the method has not been used to discriminate between sawfly taxa yet, it probably has a great value for differentiation of externally very similar sawfly species which have blade-like ovipositors and relatively complex wing venation with many potential landmarks for taxonomic purposes.

Naturally, molecular data could also be helpful for species delineation and identification in the *E. longicornis* group. Nonetheless, one must bear in mind that in cases of recently diverged groups, different markers can give contradictory results about species phylogeny because of incomplete lineage sorting and/or hybridizations (e.g. Degnan & Rosenberg 2009; Linnen & Farrell 2007, 2008; Maddison 1997; Zachos 2009). Therefore we also performed simultaneous morphological and molecular analyses to verify species delimitation and to aid identification of the *longicornis*-group species.

The primary aim of this study was to clarify the taxonomy of the *Empria longicornis* species group. Specimens from different collections (institutional and private), including the type material of eight species, were subjected to traditional and geometric morphometric analyses. DNA (mitochondrial and nuclear) sequence data of specimens from different geographical regions were used. DNA fragments sequenced were one continuous mitochondrial region (full COI, one complete, and two incomplete tRNAs) and two nuclear fragments (ITS1 and ITS2) within the rRNA locus. *Ex ovo* rearings were carried out for common, taxonomically most difficult species obtained alive in order to study their larvae, to verify the host plants, and to ensure that males of the species are correctly associated with conspecific females. Though the approach is more laborious and time consuming for the univoltine species as in the present study, it can be recommended as a complementary or an alternative to analyses of molecular markers, at least if: 1) the larval stages, host plants, and the males of some species are only partially or ambiguously known; 2) females are host specific, oviposit in captivity, and can be more reliably identified than the males; 3) imagines can be reared from larvae with reasonable success rate; 4) unfertilized females produce male progeny.

Material and methods

Specimen collection and deposition. Imagines and larvae were collected mostly by sweeping nets. Insects were killed in a freezer or in ethanol. Some of the females were provided water for drinking and kept alive for ovipositing experiments and *ex ovo* rearing. Pinned specimens studied are from the following institutional collections:

BMNH	The Natural History Museum [formerly British Museum (Natural History)], London, United Kingdom (G. Broad, S. Ryder, N. Springate);
HNHM	Hungarian Natural History Museum, Budapest, Hungary (S. Csősz, L. Zombori);
NHRS	Naturhistoriska Riksmuseet, Sektionen för entomologi, Stockholm, Sweden (H. Vårdal);
NSMT	National Museum of Nature and Science, Tokyo, Japan (A. Shinohara);
RSME	National Museums of Scotland, Edinburgh, United Kingdom (R. Lyszkowski);
SDEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany (A. Taeger, S.M. Blank, A.D. Liston);
SIZ	I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kiev, Ukraine (I.N. Pavlusenko);
TUZ	Zoological Museum of the University of Tartu, Estonia.
USNM	National Museum of Natural History, Smithsonian Institution, Washington DC, USA (D.G. Furth, D.R. Smith);

UUZM	Uppsala University, Museum of Evolution, Zoology Section, Uppsala, Sweden (H. Mejlön);
ZISP	Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (S.A. Belokobylskij, A.G. Zinovjev);
ZMH	Zoological Museum, Division of Entomology, Helsinki, Finland [including the coll. of former DABUH = Department of Applied Biology, University of Helsinki, Finland] (P. Malinen);
ZML	Museum of Zoology and Entomology, Lund University, Lund, Sweden (R. Danielsson);
ZMUC	Zoological Museum of the University, Copenhagen, Denmark (L. Vilhelmsen).

Specimens from the private collections of Stephan M. Blank, Erik Heibo, Andrew Liston, Jan Macek, Ad Mol, Guy T. Knight, Andreas Taeger, and of the first two authors were also studied. All the specimens studied are listed in the tab delimited file (http://empriini.myspecies.info/files/imports/long_group_spec.txt). The new species name is registered in ZooBank (<http://www.zoobank.org/>).

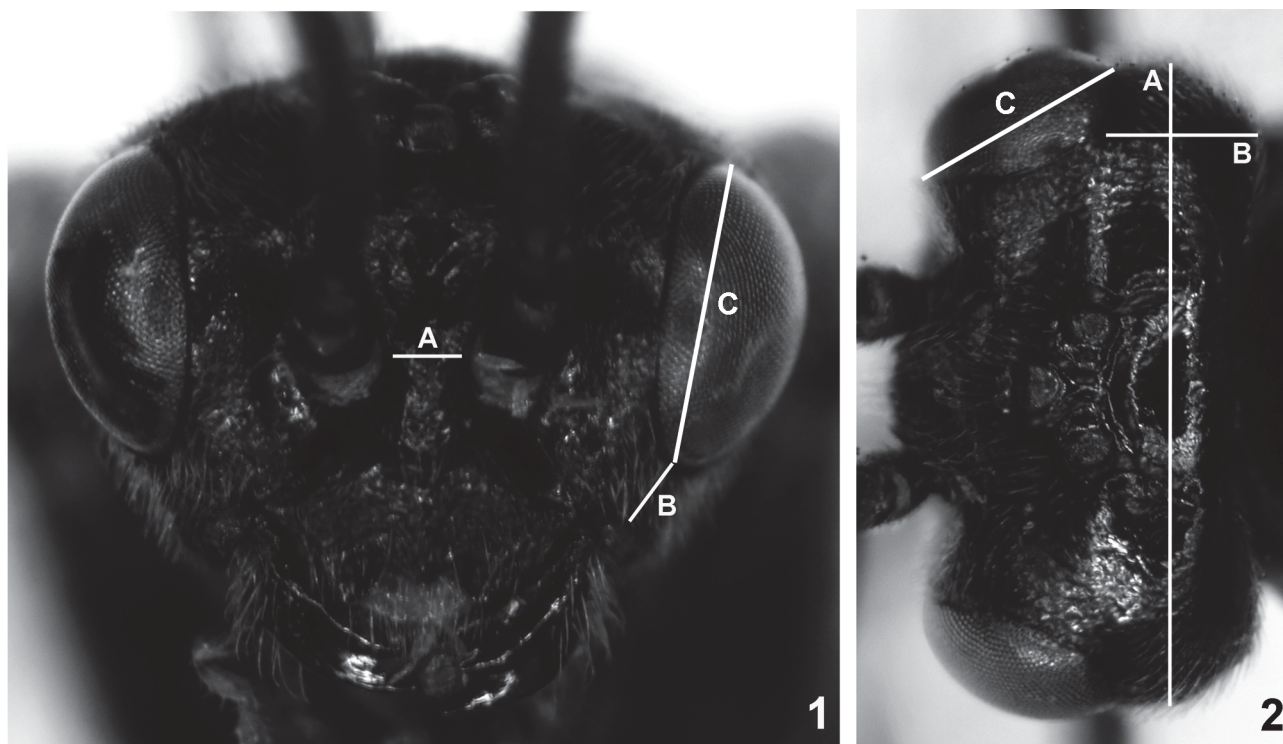
Rearing of larvae and imagines. To study larval morphology, determine the host plants, and to confirm species identity of males, rearing of larvae was carried out indoors in 2004–2010. Larvae and imagines were mostly reared *ex ovo*, but some field collected larvae were also used. Host plants were planted in plastic flower pots filled with soil. To ensure that the hosts were without previously-laid sawfly eggs, they were planted before the first adults appeared or, if planted later, they were carefully inspected. Newly hatched first instar larvae were moved to Petri dishes, each containing 1–4 larvae. Larvae were fed by fresh leaves (replaced after every 1–2 days). In few cases, additional species of Rosoideae, other than the host, were used to feed the larvae. Shed cuticles of all larval instars were preserved. If possible, at least one specimen of all instars was preserved in ethanol (70–80% or 96%). When the larvae ceased feeding and moulted to prepupal stage, they were moved to glass jars or plastic vials, which contained sterilized moist sand, sphagnum moss, and pieces of raspberry and other plant stems. The jars and vials with prepupae were taken in a basement for overwintering (minimum temperature about 3 °C). During February–March, prepupae were left outside on a balcony (where temperature fell below freezing, but not below -10 °C) for a few days and then taken step by step to room temperature (the first step of applying freezing temperatures was not always implemented).

We also conducted preliminary ovipositing experiments to examine the range of host plants of different species. There were two kinds of experiments: 1) to examine the plant preference by females, two or three different plant species were offered simultaneously, or 2) the plants were offered separately until female started to lay eggs. Females were kept with the plant(s) from one to several days or until they died (up to 1–2 weeks).

Morphological methods and analyses. Imaging methods. Living larvae were photographed using a digital camera (separately or attached to a stereomicroscope). To conduct geometric morphometric (GM) analyses, ovipositors and penis valves were photographed using a digital camera attached to a microscope and the extended focal imaging (EFI) technique was mostly applied. Set of images taken along the z axis, where each separate image had only some parts in focus, were combined to create single composite digital image with all parts in focus using the program CombineZ 4.6 or CombineZM (Alan Hadley; <http://www.hadleyweb.pwp.blueyonder.co.uk/index.htm>). If the study object did not fit to single image, the object was photographed in 2–4 partially overlapping parts and a single image covering the whole object was created using the plugin MosaicJ (Thévenaz & Unser 2007) implemented in ImageJ version 1.36b or 1.39u (Wayne Rasband; <http://rsb.info.nih.gov/ij/>) for multiple image alignment (MIA).

Morphometric analyses and measurements. Because antennal length appeared sufficient for species discrimination in the *longicornis*-group, maximal lengths and breadths of flagellomeres were measured using a stereomicroscope with the measuring scale. In some cases, the following distances on the head capsule were also measured: head length behind the compound eye in dorsal view (head positioned so posterior margins of lateral ocelli and compound eyes aligned, Fig. 2), length and height of the compound eye (Figs 1–2), between toruli, and the minimal ventro-ocular distance (Fig. 1). Breadth of the head capsule behind compound eyes (Fig. 2) was measured to account for size differences between specimens. Throughout this paper, any reference to **relative size** means linear size of a structure relative to **head breadth**, unless otherwise stated. Measurements of all the specimens (111 females and 181 males) are available in the tab delimited file (http://empriini.myspecies.info/files/imports/long_group_morph.txt). Although ovipositors of different species could be distinguished through differences in the structure of serrulae, the morphometric analyses were also used to find additional characters for species discrimination. To dissect the penis valves, genital capsules were separated from the specimen and macerated in KOH or

NaOH (10–15%) for 16–24 hours at room temperature, or treated with proteinase K using High Pure PCR Template Preparation Kit (Roche, Mannheim) and following manufacturer's protocol. Dissected penis valves and ovipositors were mounted between rectangular cover slips in euparal or glycerine, photographed and pinned with corresponding specimens as slide preparations or glued on piece of paper. Images of penis valves and the valvulae 1 of ovipositors were used to measure some linear distances (Figs 9–10) and to carry out GM analyses. Linear distances were measured using the program ImageJ version 1.36b or 1.39u. For GM analysis, landmarks shown in Figs 9 and 11 were chosen. As landmarks we chose points which were 1) well definable, 2) homologues across specimens, and 3) covered as much area as possible of the object. To minimize deformations of the penis valves under cover slips, supportive plasticine was stuck between glass slide and a cover slip corners. However, this method left sometimes too much space for penis valves to rotate around their antero-posterior axes, precluding the use of two-dimensional GM methods. Some of the potential landmarks on penis valves were excluded because of deformations. It was unnecessary to use plasticine in case of ovipositors, but basal half of the valvula 1 tended to bend sometimes, causing some of the middle annuli to overlap partially with each other. Because of such distortions, only apical part of valvula 1 was analyzed with GM methods.



FIGURES 1–2. *Empria japonica* n. sp.: 1, head in anterior view (A, minimal distance between toruli, B, minimal ventro-ocular distance, C, maximal height of the compound eye); 2, head in dorsal view (A, head breadth, B, minimal distance between the compound eye and the occipital carina = head length behind the compound eye = head length, C, maximal length of the compound eye).

The landmarks of valvula 1 were placed in three rows (Fig. 11): dorsal landmarks between adjacent annuli, middle landmarks at the pores of medial sensilla, and ventral landmarks at dorso-basal part of serrulae. There were fewer dorsal landmarks compared to middle and ventral ones, because the boundaries between the apical-most annuli were more difficult to recognize than those at the basal annuli. Two distances were measured at the base of valvula 1 (Fig. 10): “length of annulus”, defined as the distance between dorso-basal parts of two basal-most serrulae, and “height of annulus”, defined as the distance from dorso-basal part of the second (from the base of valvula 1) serrula to the dorsal joint between two basal-most serrulae-bearing annuli.

Landmarks were digitized using tpsDig 2.12 (Rohlf 2006–2008). Some of the landmarks could not be precisely located (Fig. 9) and they were defined as semilandmarks (sliders) using tpsUtil 1.4 (Rohlf 2005–2008). Digitized landmark data were analyzed using tpsRelw 1.45 (Rohlf 2007). Graphics were prepared using R Stats Package (R Development Core Team 2009). All of the measured and analyzed images are deposited in the Morphbank database (<http://www.morphbank.net/>).

Molecular methods and analyses. Species and the specimens used in molecular analyses are listed in the tab delimited file (http://empriini.myspecies.info/files/imports/long_group_spec.txt). DNA was extracted and purified with High Pure PCR Template Preparation Kit (Roche, Mannheim) according to the manufacturer's protocol and stored at -20 °C for later use. In the case of larvae, whole trunk or thorax (and often also head) was used; if the specimen was fully or nearly fully grown, the cleaned trunk cuticle was mounted in euparal after DNA extraction. In the case of imagines, thorax muscles, a leg, or, as suggested by Knölke *et al.* (2005), genitalia were used. One mitochondrial and two nuclear regions were used in phylogenetic analyses. Primers used to amplify and sequence the full COI gene (and two partial and one complete adjacent tRNA genes) are listed in Table 2. When the amplification of the full COI gene failed, the region was amplified in three or four slightly overlapping fragments (Table 2). Of the nuclear DNA, ITS1 and ITS2 were amplified and sequenced (Table 2). Although amplification of ITS1 was not problematic, sequencing proved to be more difficult, possibly because of high GC content and the presence of repetitive regions. In many cases, a middle region about 400 bp long could not be determined (all specimens of *E. alector*, most of *E. basalis* and many of *E. tridens*). For this reason, two new internal primers were designed, EmpITS1F and EmpITS1R (Table 2). EmpITS1R might be universal for *Empria* and *Monsoma*, while EmpITS1F might not always work. These internal primers enabled amplification of shorter ITS1 fragments if amplification of the full sequence failed. The shortest sequence (<300 bp) could be obtained using primers EmpITS1F and EmpITS1R, which was useful to get at least some molecular data for older air-dried museum specimens. To improve sequencing quality of ITS1, combinatorial enhancer solution (CES) (Ralser *et al.* 2006) was sometimes used. PCR reactions were carried out in a total volume of 20 µl containing 4–100 ng of genomic DNA, 5 pmol of primers, 0.2 mM dNTP mixture (Fermentas, Vilnius), 1U of Advantage 2 PCR buffer and 1U of Advantage 2 Polymerase mix (BD Biosciences, San Jose). The PCR programme consisted of an initial denaturing step at 95°C for 1 min, followed by 35–45 cycles of 20 s at 95°C, 30 s at 45–65°C depending on a primer set used and 50–70 s (depending on the amplicon size) at 68°C; the last cycle was followed by a final 7 min extension step at 68°C. The full COI was amplified using the touchdown profile, in which the annealing temperature decreased from 55°C to 45°C by 0.5°C every cycle, and the final 24 cycles had annealing at 45°C (other PCR steps were the same as described above). PCR product was purified with shrimp alkaline phosphatase (or FastAP) and exonuclease I. 1U of both enzymes (Fermentas, Vilnius) were added to 10 µl of PCR solution and incubated for 27 min (or 10 min in case of FastAP) at 37°C, followed by 15 min at 80°C. The purified PCR product was directly used for sequencing. DNA cycle sequencing was performed by using DYEnamic ET Terminator Cycle Sequencing kit (GE Healthcare, Chalfont St Giles) or BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City) with the addition of CES (Ralser *et al.* 2006) in some cases. 33 cycles (15 s at 95°C, 15 s at 45–55°C and 60 s at 60°C) were performed on Gene Amp PCR System 2700 (Applied Biosystems, Foster City) or TProfessional Thermocycler (Biometra, Göttingen) in a total volume of 10 µl. To obtain unequivocal sequences, both sense and antisense strands were sequenced, using the primers listed in Table 2. Sequences were resolved on ABI PRISM 377 or 3130xl automated DNA sequencers (Applied Biosystems, Foster City). Based on sequence chromatograms, consensus sequences of both strands were created for every marker and specimen. Few ITS sequences of females were polymorphic for length and in those cases heterozygous insertions/deletions (indels) were reconstructed using the program Indelligent v.1.2 (Dmitriev & Rakitov 2008), available at <http://ctap.inhs.uiuc.edu/dmitriev/indel.asp>. Ambiguous positions (i.e. double peaks in chromatograms of both strands) due to heterozygosity or intragenomic variation were coded using IUPAC symbols.

Sequences reported here have been deposited in the GenBank (NCBI) database (accession numbers HM177266–HM177415 and HQ412768–HQ412770).

COI and tRNA sequences were aligned manually. Delimitation and alignment of tRNA sequences were aided by secondary structure, which was detected using tRNAscan-SE 1.21 (Lowe & Eddy 1997). Among COI sequences only *Monsoma pulveratum* (Retzius, 1783) contained insertion of three base pairs, which was located by translating nucleotides into amino acids (using invertebrate mitochondrial genetic code). Boundaries of ITS2 sequences were identified with ITS2-Annotation tool (which uses HMMer; Eddy 1998) available through the ITS2 Database (Selig *et al.* 2008). ITS sequences of the *E. longicornis* group species, species, *E. immersa* (Klug, 1818), and *E. fletcheri* (Cameron, 1878) were aligned manually. Substitution models for DNA alignments were selected using FindModel (Tao *et al.* 2008).

TABLE 2. Properties of primers used in this study including annealing temperatures for DNA marker amplification (PCR) and cycle sequencing (CS).

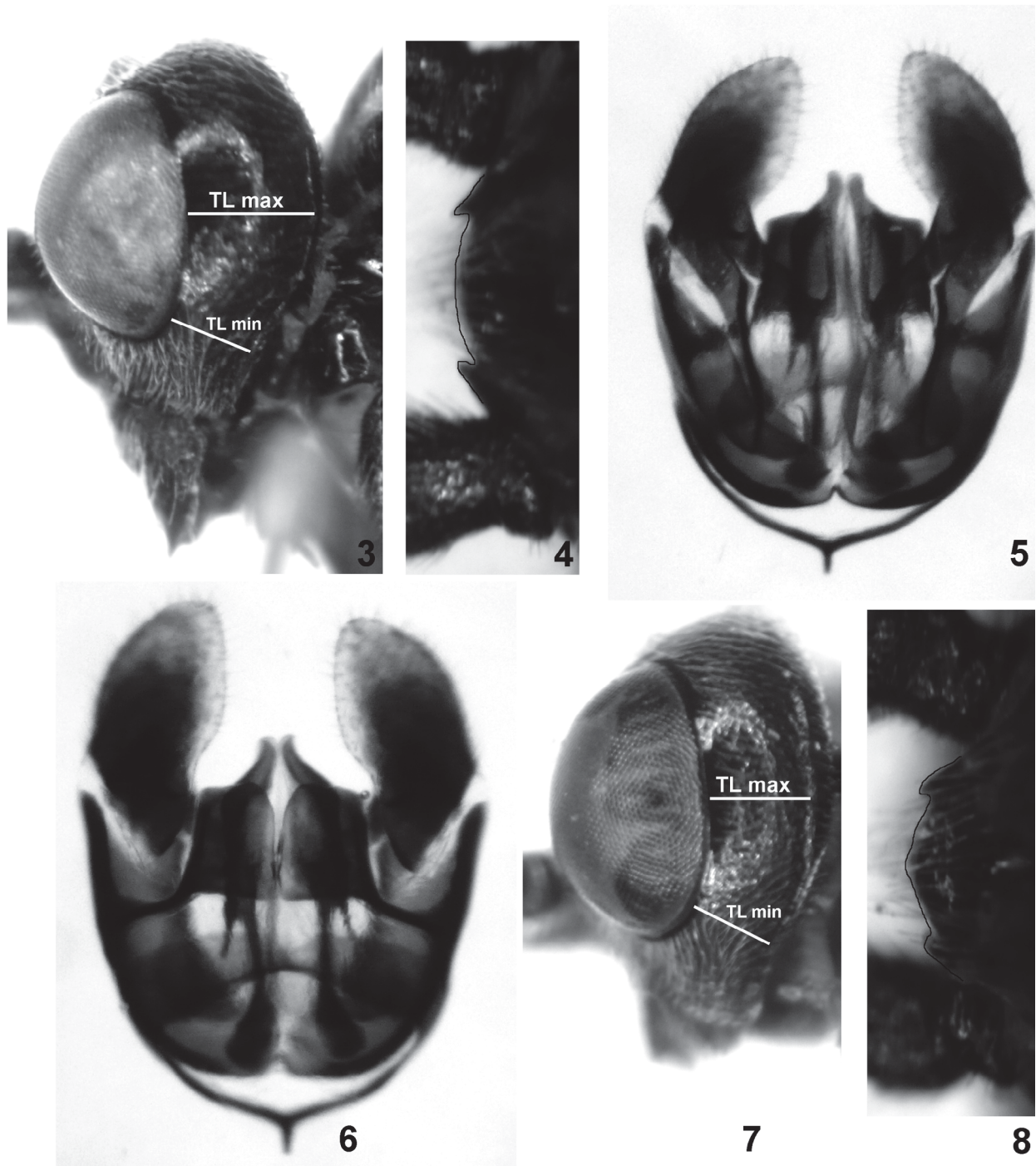
Primer	Direction	Primer sequence (5' to 3')	Primer location	PCR (°C)	CS (°C)	Reference
TW-J1301	Forward	GTAAWTAAACTAA-TARCCTTCAAAA	tRNA-Cys	49	49	Simon <i>et al.</i> (2006)
TY-J1460	Forward	TTTACAATTTATCGC-CTAAAMTTCAGCC	tRNA-Tyr	47	47	This study
C1-J1718	Forward	GGAGGATTTGGAAAY-TGAYTAGTWCC	COI	49	49	Nyman <i>et al.</i> (2006)
C1-J1751 (Ron)	Forward	GGATCACCTGATATAG-CATTCCC	COI	46	46	Simon <i>et al.</i> (1994)
C1-N1760	Reverse	GGTARAAATCAR-AATCTTATATTAT	COI	49	49	This study
C1-J2186	Forward	CAACAYYTATTTT-GATTTTTTGGWCA	COI	48	48	This study
C1-N2191 (Nancy)	Reverse	CCCGGTAAAAAT-TAAAATATAAACTTC	COI	46	46	Simon <i>et al.</i> (1994)
C1-J2435	Forward	ACAGGAAT-TAAAATTTTGTAGRTG	COI	48	48	This study
A2590	Reverse	GCTCCTATTGA-TARWACATART-GRAAATG	COI	49	49	Normark <i>et al.</i> (1999)
TL2-N3018 (1)	Reverse	CCATTGCATTTTCT-GCCAT	tRNA-Leu	(2)	47	This study
C2-N3083	Reverse	TAAAARTTWGYTCAT-GTTGTCAT	COII	48	48	This study
CAS18sF1	Forward	TACACACCGC-CCGTCGCTACTA	18S rRNA	65	60	Ji <i>et al.</i> (2003)
CAS5p8sB1d	Reverse	ATGTGCGTTTCRAAAT-GTCGATGTTCA	5.8S rRNA	65	60	Ji <i>et al.</i> (2003)
EmpITS1F	Forward	GAACGWCCTAACGGC-CGGTRT	ITS1	60	60	This study
EmpITS1R	Reverse	TCGTGCAGAGCGCCG-GGTCCGA	ITS1	60	60	This study
CAS5p8sFc	Forward	TGAACATCGACATTTY-GAACGCACAT	5.8S rRNA	62	60	Ji <i>et al.</i> (2003)
CAS28sB1d	Reverse	TTCTTTTCCTCCGCTT-ATTRATATGCTTAA	28S rRNA	62	60	Ji <i>et al.</i> (2003)

(1), reverse complement of TL2-J3037 (Simon *et al.* 1994).

(2), not used for amplification.

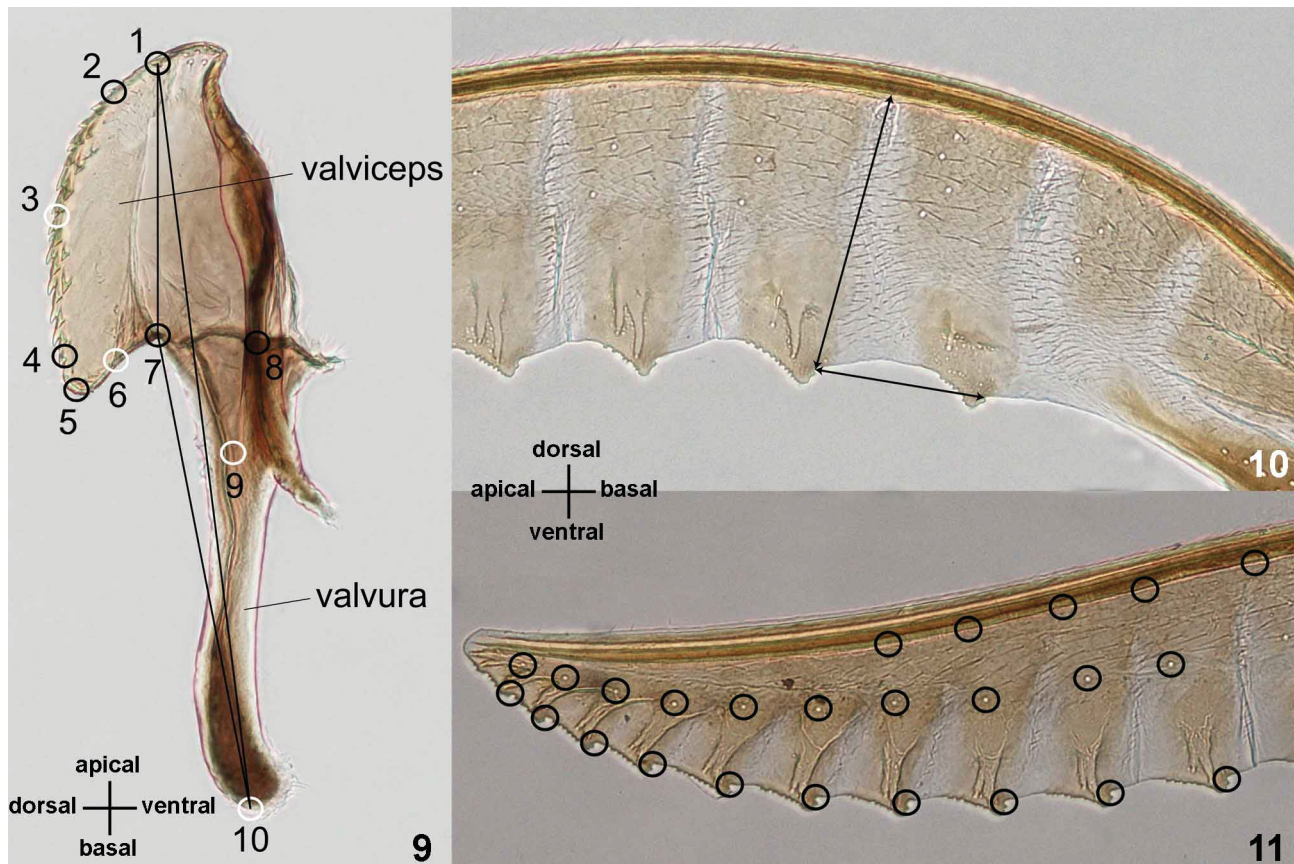
Sequence data was analyzed using the neighbour joining (NJ, with Mega 4.0; Tamura *et al.* 2007), maximum likelihood (ML, with PhyML 3.0.1 at <http://www.atgc-montpellier.fr/phyml/>; Guindon & Gascuel 2003; and Treefinder version of October 2008; Jobb 2008; Jobb *et al.* 2004), and Bayesian methods (MrBayes 3.1.2, Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; and BALi-Phy, Suchard & Redelings 2006). Tree topologies were tested in Treefinder version of October 2008 (Jobb 2008; Jobb *et al.* 2004). In PhyML nearest neighbor interchanges (NNI) and subtree pruning and regrafting (SPR) were always used to estimate tree topologies (i.e. using the extensive tree search option). Robustness of reconstructed trees was estimated with 500 (ML) or 1000 (NJ) bootstrap replicates. Prior to analyses using Bayesian or maximum likelihood methods, duplicate sequences (including those which were duplicates after the exclusion of ambiguous positions) were removed to save computation time. In case of MrBayes, we used a threshold of 0.01 for the average standard deviation of split frequencies between the two runs as a diagnostic for convergence. Because this threshold was achieved after 0.2–0.5 million

generations, one million generations and burnin 25% or 50% was considered sufficient. Because of numerous indel events several regions of ITS genes proved to be difficult to align, therefore BALi-Phy was used to include information from these regions and also indel events. This program has implementations to handle difficult-to-align sequences and to include indels in phylogenetic analyses but it is computationally intensive. To enhance the speed of calculation, conserved regions of ITS genes were fixed in the analysis with BALi-Phy. Two to four independent runs consisting of 20,000-200,000 generations with burnin 10–50% were analyzed. Tracer 1.4 (<http://beast.bio.ed.ac.uk/Tracer>) was used to ensure the stationary of output values. *Monsoma pulveratum* or species belonging to the *E. immersa* group (if more distant outgroups were excluded) were used to root the phylogenetic trees.



FIGURES 3–6. *Empria japonica* n. sp.: 3, head in lateral view (TL = temple length); 4, frontal crest in dorsal view; 5, genital capsule in dorsal view; 6, genital capsule in ventral view.

FIGURES 7–8. *Empria longicornis* Thomson, 1871: 7, head in lateral view (TL = temple length); 8, frontal crest in dorsal view.



FIGURES 9–11. Landmarks (LMs) used in geometric morphometric (GM) analyses of penis valves and ovipositors, and some linear measurements: 9, landmarks (black circles: 1, base of the dorso-apical tooth of valviceps; 2, apical-most part of teethrow of valviceps; 4, basal-most part of teethrow of valviceps; 5, tip of dorso-basal part of valviceps; 7, dorsal joint between valvura and valviceps; 8, ventral joint between valvura and valviceps), semi-landmarks (white circles: 3, midway between landmarks 2 and 4; 6, midway between landmarks 5 and 7; 9, junction of valvular duct and ventral edge of penis valve; 10, tip of basal part of valvura), and linear measurements (LM1–LM7 as the “length of valviceps”, LM7–LM10 as the “length of valvura”, and LM1–LM10 as the “length of penis valve”) of penis valves; 10, linear measurements of the basal-most serrula-bearing annulus of the valvula 1 (horizontal arrow, distance between dorso-basal parts of the serrulae; vertical arrow, distance from dorso-basal part of the serrula to the dorsal joint between two annuli); 11, landmarks (black circles) of the valvula 1.

Results

Morphology

Females. Results of GM analyses of the *longicornis*-group species (except *E. mongolica*) revealed that *Empria alpina*, *E. minuta* (= *E. gussakovskii* auct., see the taxonomic results below), and *E. loktini* could not be distinguished from *E. basalis* (details not shown), but serrulae in these species are clearly different from *E. basalis* and from each other as well (Figs 36, 38–39, 44). For the sake of clarity, these three species were excluded from subsequent analyses. Results of the GM analyses of valvula 1 separated the remaining five species into three groups: *E. alector*, *E. basalis*, and *E. tridens*–*E. longicornis*–*E. japonica* (not shown). Although only three specimens of *E. japonica* were measured, this species can be distinguished from *E. tridens* and *E. longicornis* by having the longest basal-most annulus of valvula 1, both on the absolute and relative scale: exceeds 0.1 mm (relative length exceeds 0.065) in *E. japonica*, less than 0.1 mm (relative length less than 0.065) in other two species. Furthermore, *E. japonica* could be distinguished quite well from *E. tridens* through the relative height of basal-most annulus (Fig. 10) (>0.115 in *E. japonica*, <0.108 in *E. tridens*).

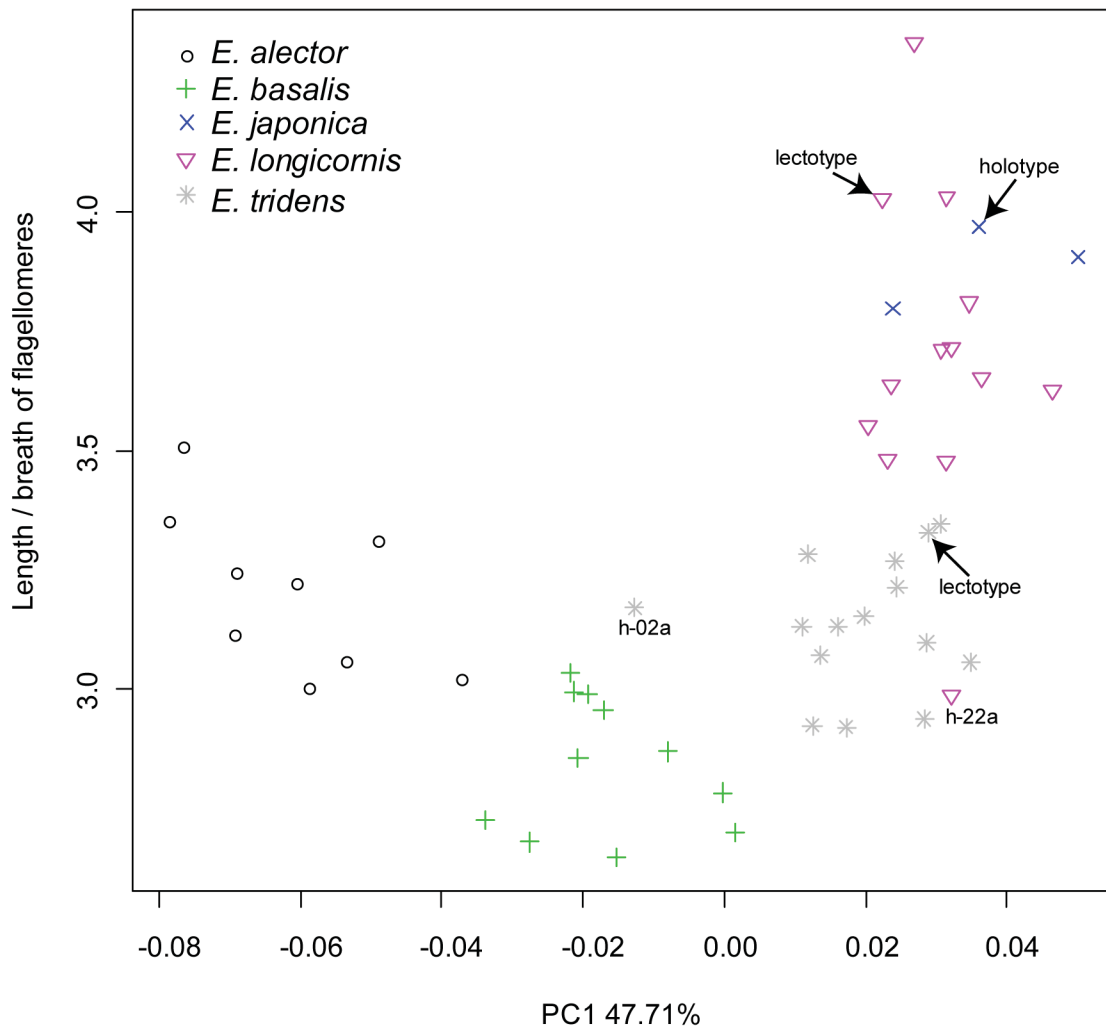


FIGURE 12. GM analyses (25 landmarks) of valvula 1 and the relative length of flagellum of 5 species. First principal component (PC1) is on the x-axis, relative length of flagellum (sum of lengths of individual flagellomeres divided by sum of breadths) on the y-axis. Specimens discussed in the text, are indicated by their ID numbers.

Empria longicornis can be differentiated from *E. tridens* by its more robust serrulae (compare Figs 41, 46 with 42, 45) and mostly also by greater relative length of its flagellae (Figs 12–13). Specimen h-02a was placed very close to *E. basalis* by GM analyses (Figs 12–13), but according to the structure of its serrulae, the specimen is *E. tridens* (Fig. 45). Interestingly, one specimen (h-22a) classified by its ovipositor structure as *E. longicornis*, has relatively short flagellae (Figs 12–13).

Males. We successfully reared *ex ovo* males of four common species of the group (see Table 6). Such reared males of certain taxonomic identity allowed us to evaluate diagnostic characters for the males of taxonomically most problematic species and to identify all the males with reasonable confidence (including those not reared *ex ovo*). Dubious identifications are indicated with a question mark in the results of morphometric analyses. We were not able to obtain live individuals for rearing the males of less widespread *E. mongolica*, *E. alpina*, *E. loktini*, *E. minuta*, and *E. japonica*. However, identification of males of these species (except *E. minuta*) is less problematic. After the manuscript was already in review, we discovered some males (previously unknown) and a female of *E. minuta* in Malaise trap catches. Identity of one male was also confirmed by ITS sequences (not shown).

GM analyses of penis valves (except *E. mongolica* and *E. minuta*) using 10 landmarks (LMs) did not reveal such clearly distinct clusters as did the analysis of ovipositors (not shown). Nevertheless, with *E. alector* excluded, *E. basalis* can be distinguished from other species, albeit overlapping with some specimens of *E. tridens* and *E. japonica*. The situation is similar for *E. alector* if *E. basalis* is excluded. The first principal component of the GM analysis combined with the relative length of flagellum separates reasonably well most of the species, except *E. tridens*, which overlaps partially with *E. basalis* and *E. longicornis* (Fig. 14).

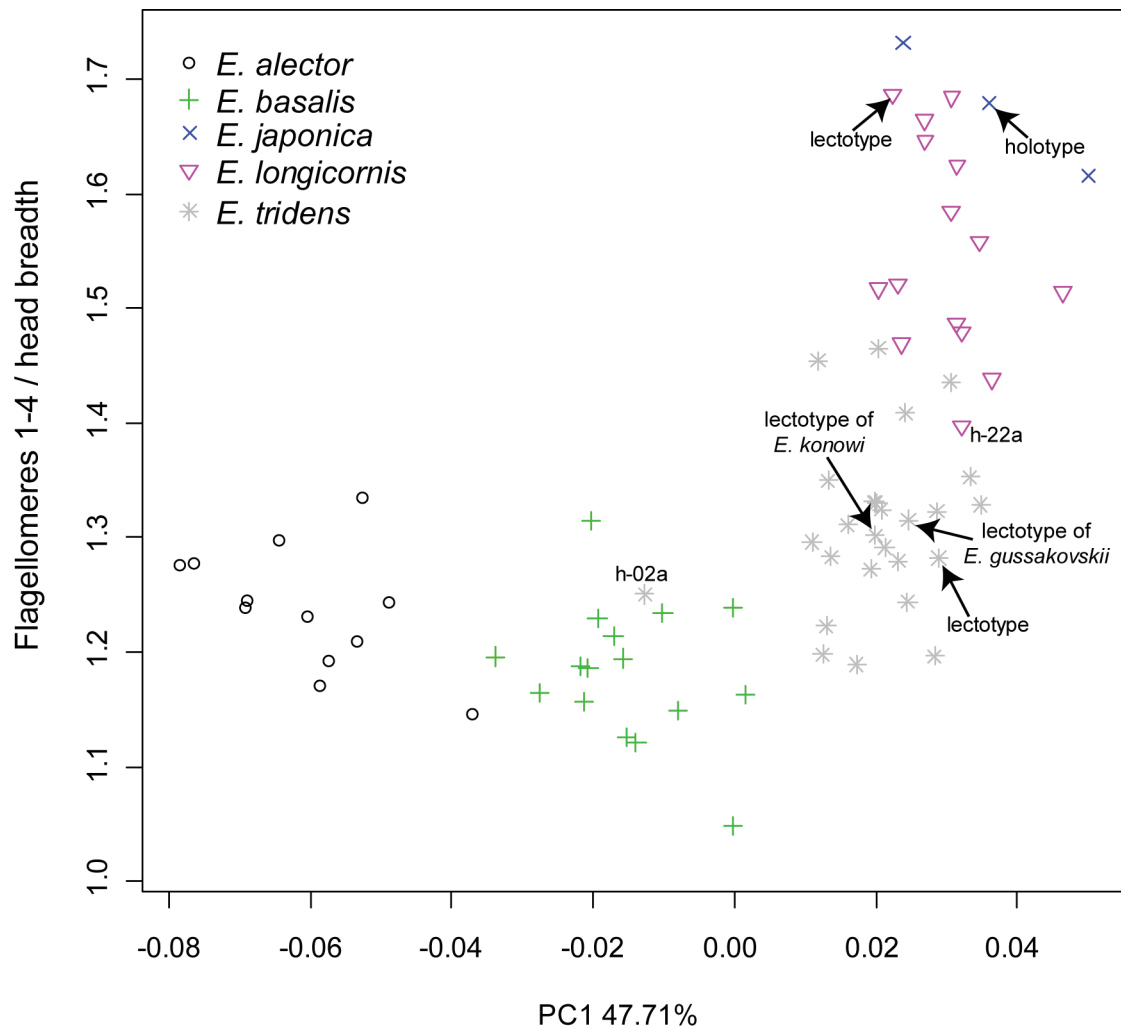


FIGURE 13. GM analyses (25 landmarks) of valvula 1 and the relative length of flagellum of 5 species. First principal component (PC1) is on the x-axis, relative length of flagellum (sum of lengths of flagellomeres 1–4 divided by head breadth) on the y-axis. Specimens discussed in the text, are indicated by their ID numbers.

Although GM analysis using 10 LMs did not fully differentiate between *E. basalis* and *E. tridens*, the two can be separated by differently shaped valviceps: in *E. basalis* the lobe of the valviceps is longer and clearly bent towards valvura, while in *E. tridens* the lobe is shorter and straighter, bending towards valvura only slightly if at all (compare Fig. 52 with 49–50). Differences between the penis valves of these two species are more evident in GM analyses using 9 LMs (LM 10 excluded, see Fig. 9) (Fig. 15). The hardly separable species-pair is *E. longicornis*–*E. tridens*. Though *E. tridens* has antennae absolutely or relatively shorter than *E. longicornis*, the structure of their penis valves should also be studied, because in *E. tridens* the valviceps is relatively longer with its baso-dorsal tip mostly rounder compared to *E. longicornis*.

Only a few males of this species group can not be identified by structural characters. For instance, a male from France (08-12, see Fig. 57), initially regarded as an undescribed species of the *longicornis*-group (GM analyses revealed also distinctness of its penis valves; see Fig. 15), was later recognized as *E. tridens* (Fig. 18) using its ITS sequence. It probably is an aberrant specimen, no other penis valves alike (08-12) were found among more than 200 studied specimens (for example the con-local specimen 08-13 collected at the same time is fairly typical *E. tridens*, see Morphbank image id=578849). Because of haploidy, aberrant males are expected to be more common than females. In the same regard, the penis valves of the specimen h-32 (Fig. 56) are distinct from *E. alector* in morphometric analyses using 10 LMs (Fig. 14), probably because of its exceptionally short valvura (valiceps to valvura ratio is 0.77, in most other cases it is 0.45–0.70). Despite its exceptionally short valvura, the specimen fits well to *E. alector* (GM analysis excluding the LM 10, not shown).

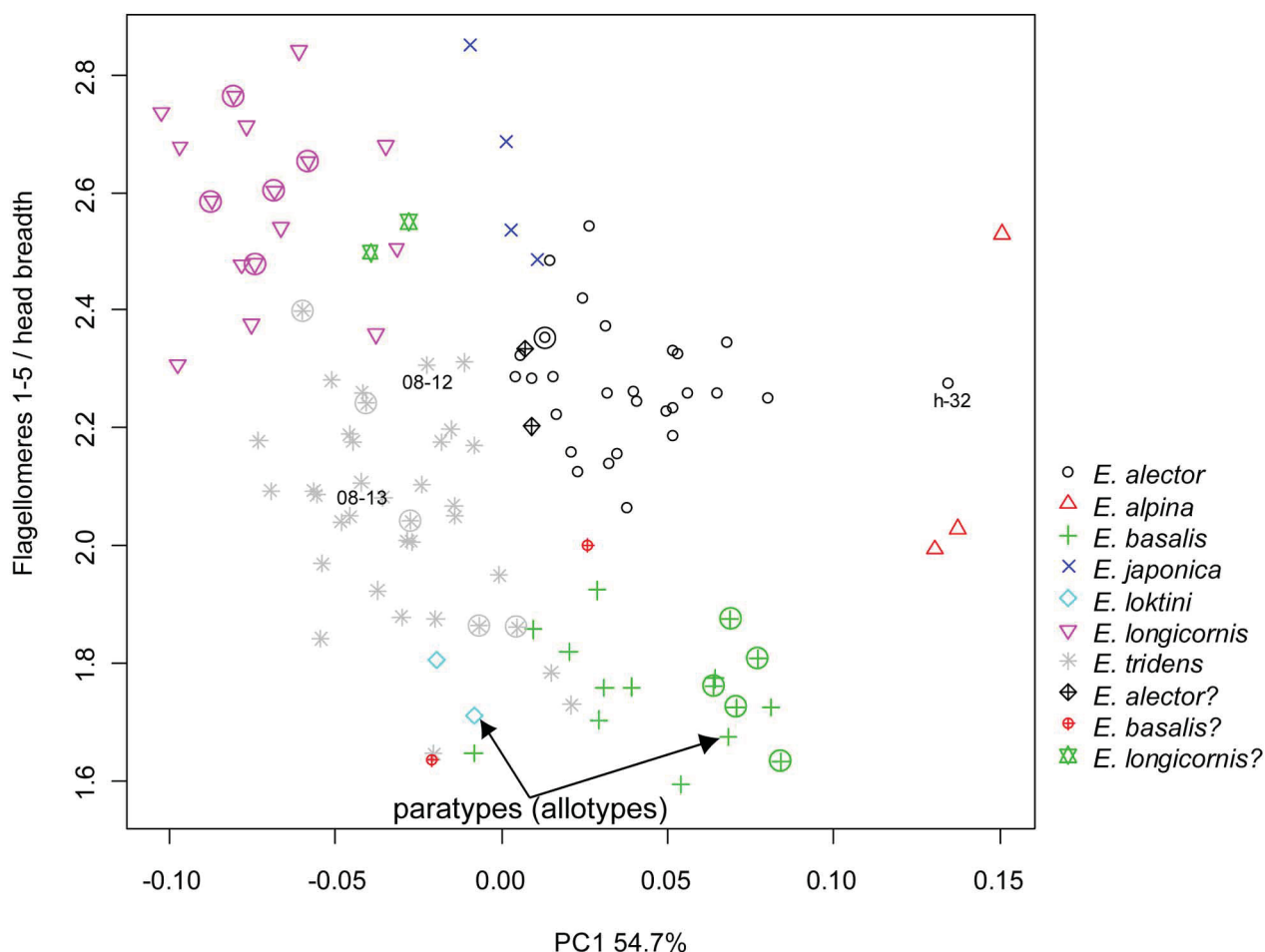


FIGURE 14. GM analyses (10 landmarks) of penis valves and the relative length of flagellum. First principal component (PC1) is on the x-axis, relative length of flagellum (sum of lengths of flagellomeres 1–5 divided by head breadth) on the y-axis. Specimens discussed in the text, are indicated by their ID numbers, and *ex ovo* reared specimens are circled.

Molecular phylogenetic analyses

Amplified ITS1 sequences (including partial 18S and 5.8S rDNA) were 967–1088 bp and ITS2 sequences (without 5.8S and 28S rDNA) 406–497 bp long, within the *E. longicornis* group 1033–1054 bp (ITS1) and 466–472 bp (ITS2).

Phylogenetic analyses of nuclear sequences. Phylogenetic analyses of ITS sequences revealed monophyly of most species with more than one specimen sampled (Figs 16, 18). Only *E. tridens*, as circumscribed here, appeared para- or polyphyletic according to this marker. Clade support values were the highest for BALi-Phy, slightly lower for MrBayes and the lowest for maximum likelihood analysis. Low bootstrap support values of maximum likelihood analysis can be explained by minor divergence of the ITS sequences within the *longicornis*-group. Lower posterior probability values (PP) of MrBayes compared to BALi-Phy are probably due to smaller number of characters analyzed (insertions and deletions are ignored in MrBayes and most other programs, while BALi-Phy takes them into account). Only BALi-Phy recovered *E. japonica* as the sister group of LTAB clade (*E. longicornis*, *E. tridens*, *E. alector*, *E. basalis*), which is apparently based entirely on indel characters, because other methods did not support this grouping neither the alternative topologies.

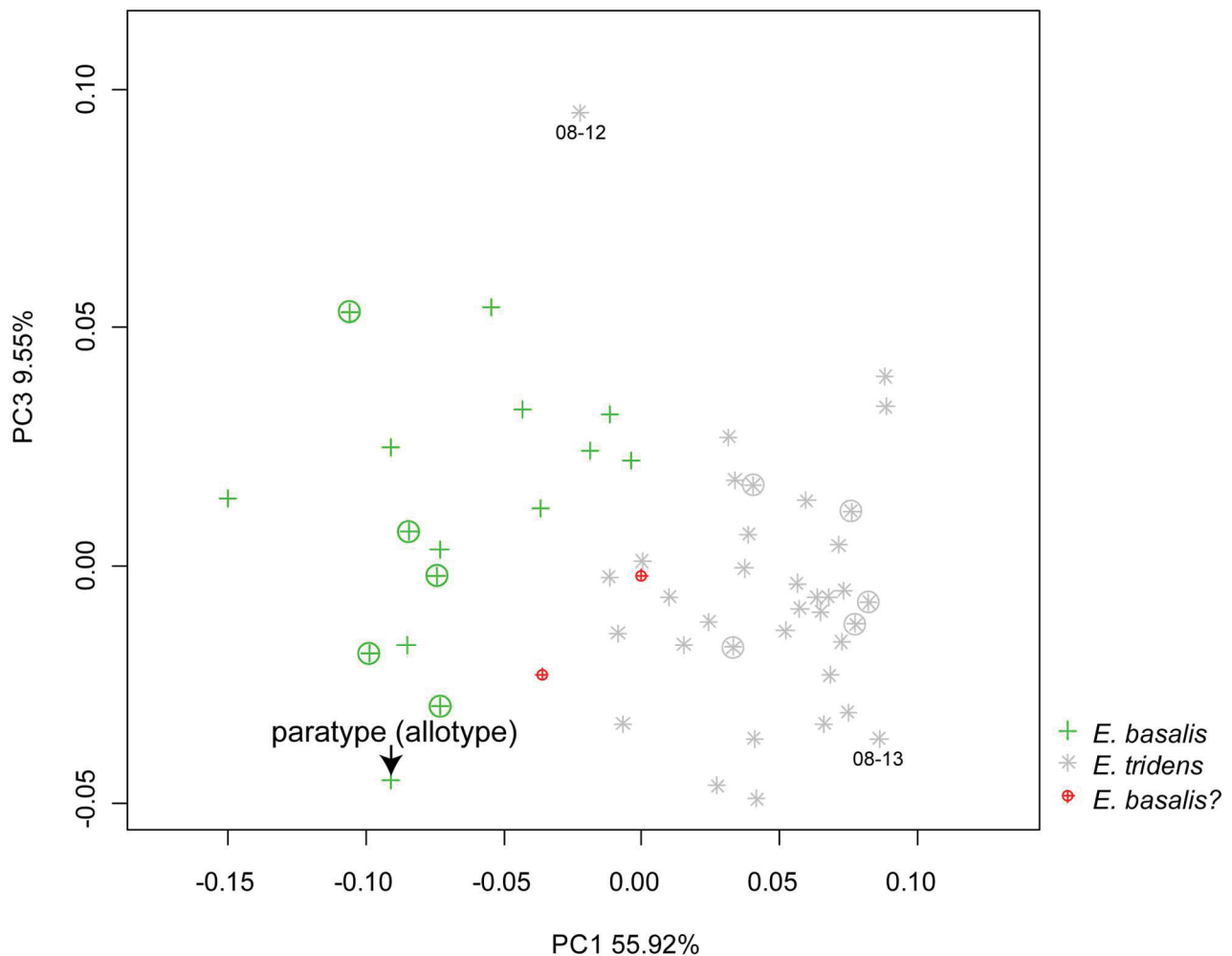


FIGURE 15. GM analyses (9 landmarks) of penis valves. First principal component (PC1) is on the x-axis, third principal component (PC3) on the y-axis. Only *E. basalis* and *E. tridens* are shown. Specimens discussed in the text, are indicated by their ID numbers, and *ex ovo* reared specimens are circled.

Because the complete ITS1 sequence of *E. minuta* was obtained only recently, the computationally most demanding phylogenetic analyses were performed also using shorter sequences. BALi-Phy analysis based on a half of the ITS1 sequence and the complete ITS2 sequence moderately supports (posterior probability 0.91) one specimen of *E. minuta* as a sister to other species of the *longicornis*-group (not shown). Analysis using about a quarter of the ITS1 sequence and the complete ITS2 sequence (not shown) supported this phylogenetic placement of *E. minuta* (two specimens) only weakly (PP=0.78). For some specimens, amplification of only short (less than 250 bp) ITS1 fragments was successful. The phylogeny reconstructed from this short region (Fig. 17) agrees to a large extent with the results based on longer alignments, but the resolution of this tree is naturally much poorer. Like in most other species (Figs 16, 18), the monophyly of *E. minuta* is also supported by ITS sequences (Fig. 17).

Two *E. tridens* specimens from Estonia and one from Japan, have ITS sequences clearly different from the others (Fig. 18; Table 3). Interestingly, these ITS sequence differences correspond with the external morphology of larvae (Table 3; see also Table 8). However, too few larvae are reared to draw any definite conclusions. Imaginal morphology does not seem to correlate with the ITS sequence variation.

Phylogenetic analyses of mitochondrial sequences. Most of the *longicornis*-group species, except *E. japonica* (n=3), were not monophyletic on the basis of mitochondrial markers analyzed (Figs 19–20). Haplotypes of different species in the group can be identical or nearly identical, but there can be also very distant haplotypes within the same species (Table 4; Figs 19–20). Though beyond the scope of this paper, we note that *E. immersa*, one of the outgroup species, is also non-monophyletic according to the mtDNA markers (Figs 19–20).

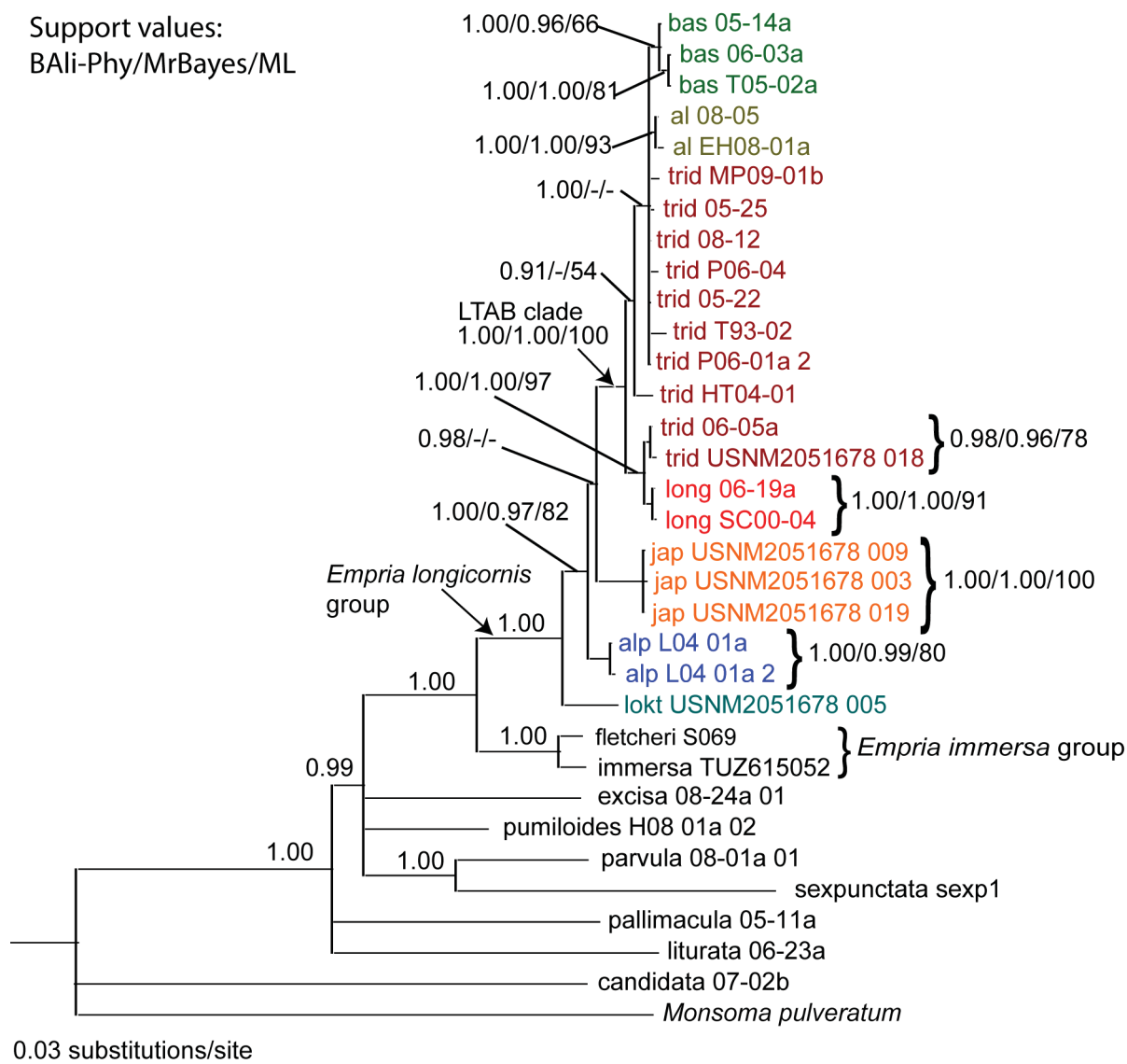


FIGURE 16. Phylogeny of ITS sequences reconstructed using BALi-Phy (GTR+4 Γ substitution model). Duplicate sequences were removed prior to analyses and are not shown. Clades with posterior probabilities (PP) less than 0.9 were collapsed. The support values shown for maximum likelihood (ML) and MrBayes analyses are from analyses based on manual alignment using *Empria immersa* group as the only outgroup. The same ML and MrBayes analyses using BALi-Phy alignment gave very similar support values. Support values less than 50% (bootstrap, ML) or 0.9 (posterior probability, MrBayes) are indicated with “-“. **al**, *E. alector*; **alp**, *E. alpina*; **bas**, *E. basalis*; **jap**, *E. japonica*; **lokt**, *E. loktini*; **long**, *E. longicornis*; **trid**, *E. tridens*.

Conflicting phylogenetic signals in nuclear and mitochondrial sequences. Inconsistencies in phylogenetic results obtained using the mitochondrial and nuclear markers were clearly evident already from a cursory examination of the alignments by eye. We tested mitochondrial and ITS tree topologies using mitochondrial or ITS alignments. Results show unambiguously that when the ITS alignment is used to test these two competing phylogenetic hypotheses, the mitochondrial topology is rejected and when mtDNA is used, the ITS topology is rejected (Table 5). Clearly, there is a significant conflict between the phylogenetic signals of the mitochondrial and ITS sequences. Thus, concatenation of nuclear and mitochondrial datasets is not justified.

Nevertheless we combined the nuclear and mitochondrial sequences to examine the behaviour of statistical support on clades. As expected, clade support values did not increase, despite more than doubling (compared to ITS only alignment) the amount of characters, rather there was a decrease in support values (not shown). Species are not monophyletic in this combined phylogeny, likely due to the conflicting signal in mitochondrial sequences (not shown).

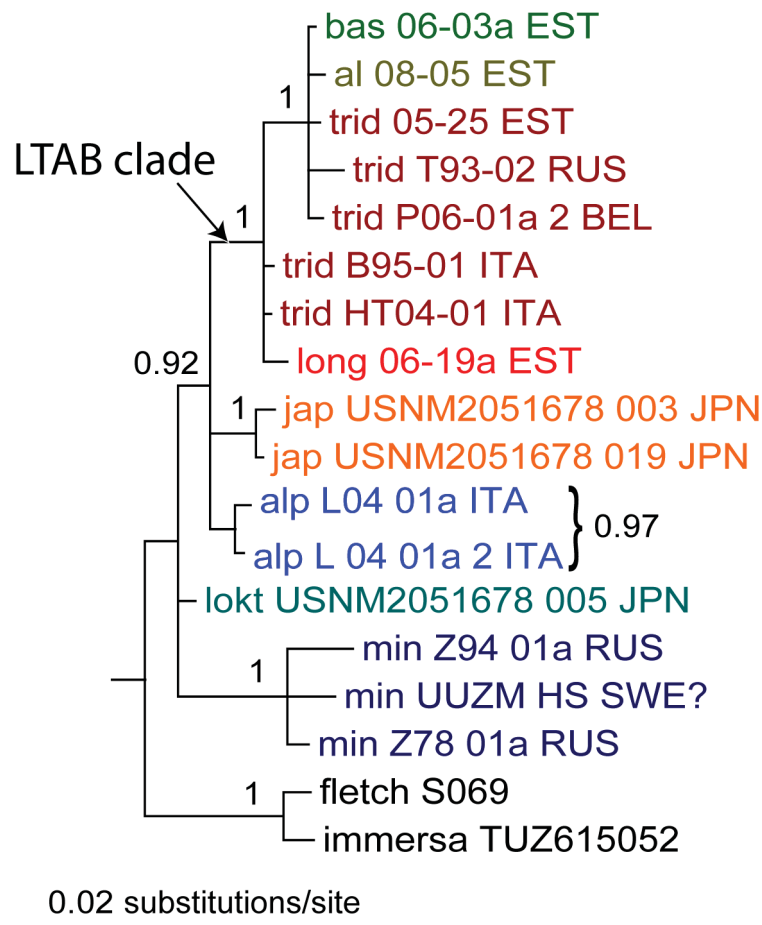


FIGURE 17. Phylogeny of about a quarter of ITS1 reconstructed using BAli-Phy (GTR+4 Γ substitution model). Duplicate sequences were removed prior to analyses and are not shown. Clades with posterior probabilities less than 0.9 were collapsed. *Empria immersa* group was used to root the tree. BEL, Belgium; EST, Estonia; ITA, Italy; JPN, Japan; RUS, Russia; SWE, Sweden. **al**, *E. alector*; **alp**, *E. alpina*; **bas**, *E. basalis*; **fletch**, *E. fletcheri*; **jap**, *E. japonica*; **lokt**, *E. loktini*; **long**, *E. longicornis*; **trid**, *E. tridens*.

TABLE 3. Comparison of two differentiable forms of *Empria tridens*.

specimen	ITS “haplotype”	Larval type	Flagellum (males)	Tegulae	Proximal part of hind tibiae
07-04a	1	1	Long	black	intermediate
MP09-01b	1	1	?	?	?
T93-02	1	?	Short	intermediate	white
06-05a	2	2	Short	whitish	white
06-06a	2	2	Short	whitish	white
USNM2051678_018	2	?	-	black	black

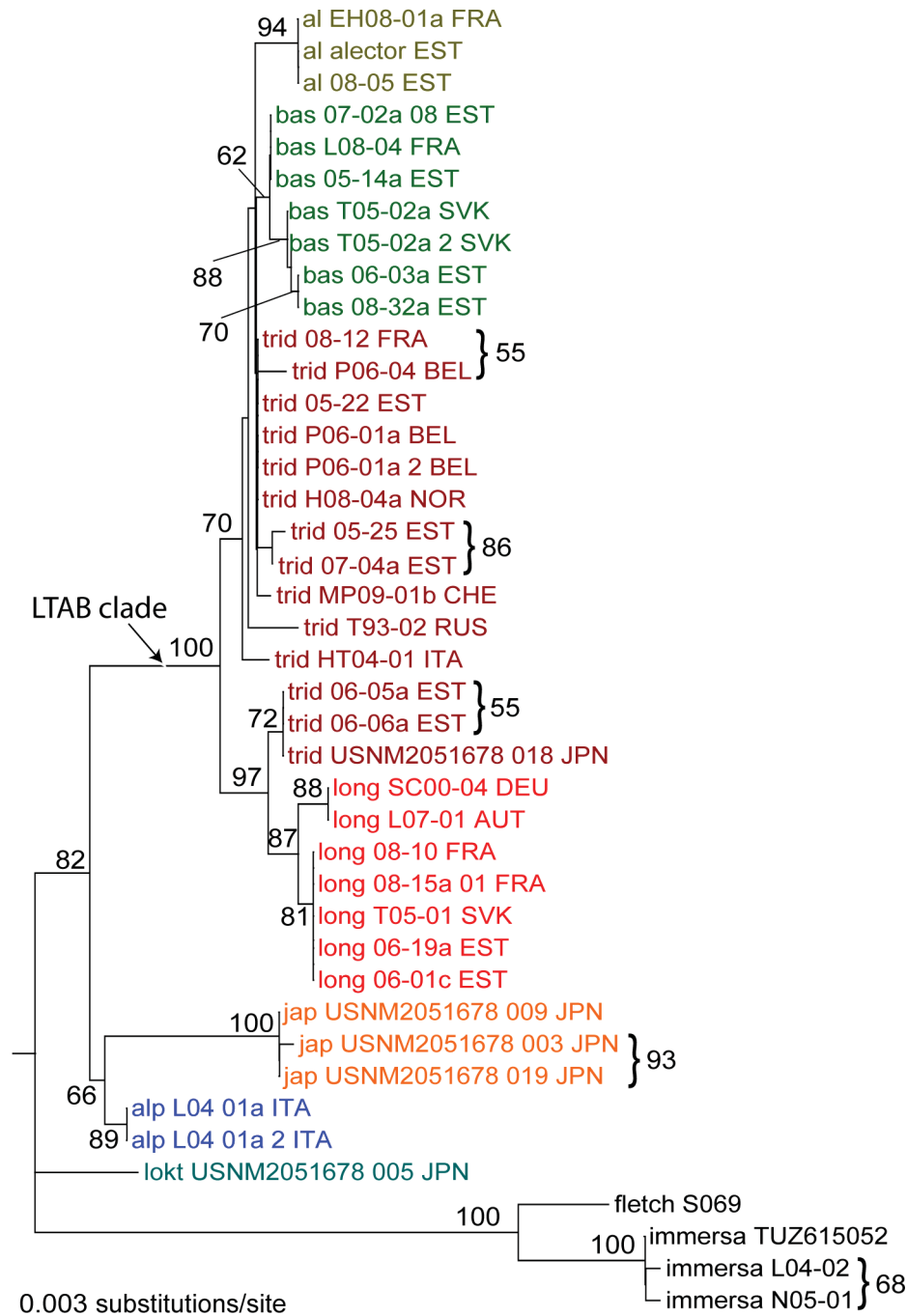


FIGURE 18. Barcoding analysis of manually aligned ITS sequences (incomplete sequences were excluded; alignment length 1348 bp). NJ tree based on Kimura two-parameter distances, pairwise deletion. Bootstrap proportions below 50% are not shown. *Empria immersa* group was used to root the tree. Barcoding analysis using the BALi-Phy MAP alignment (BALi-Phy analysis using *E. immersa* group as the only outgroup) gave nearly identical topology, the differences were not significantly (i.e. bootstrap proportions below 50%) supported. AUT, Austria; BEL, Belgium; CHE, Switzerland; DEU, Germany; EST, Estonia; FRA, France; ITA, Italy; JPN, Japan; NOR, Norway; RUS, Russia; SVK, Slovakia. **al**, *E. alector*; **alp**, *E. alpina*; **bas**, *E. basalis*; **fletch**, *E. fletcheri*; **immersa**, *E. immersa*; **jap**, *E. japonica*; **lokt**, *E. loktini*; **long**, *E. longicornis*; **trid**, *E. tridens*.

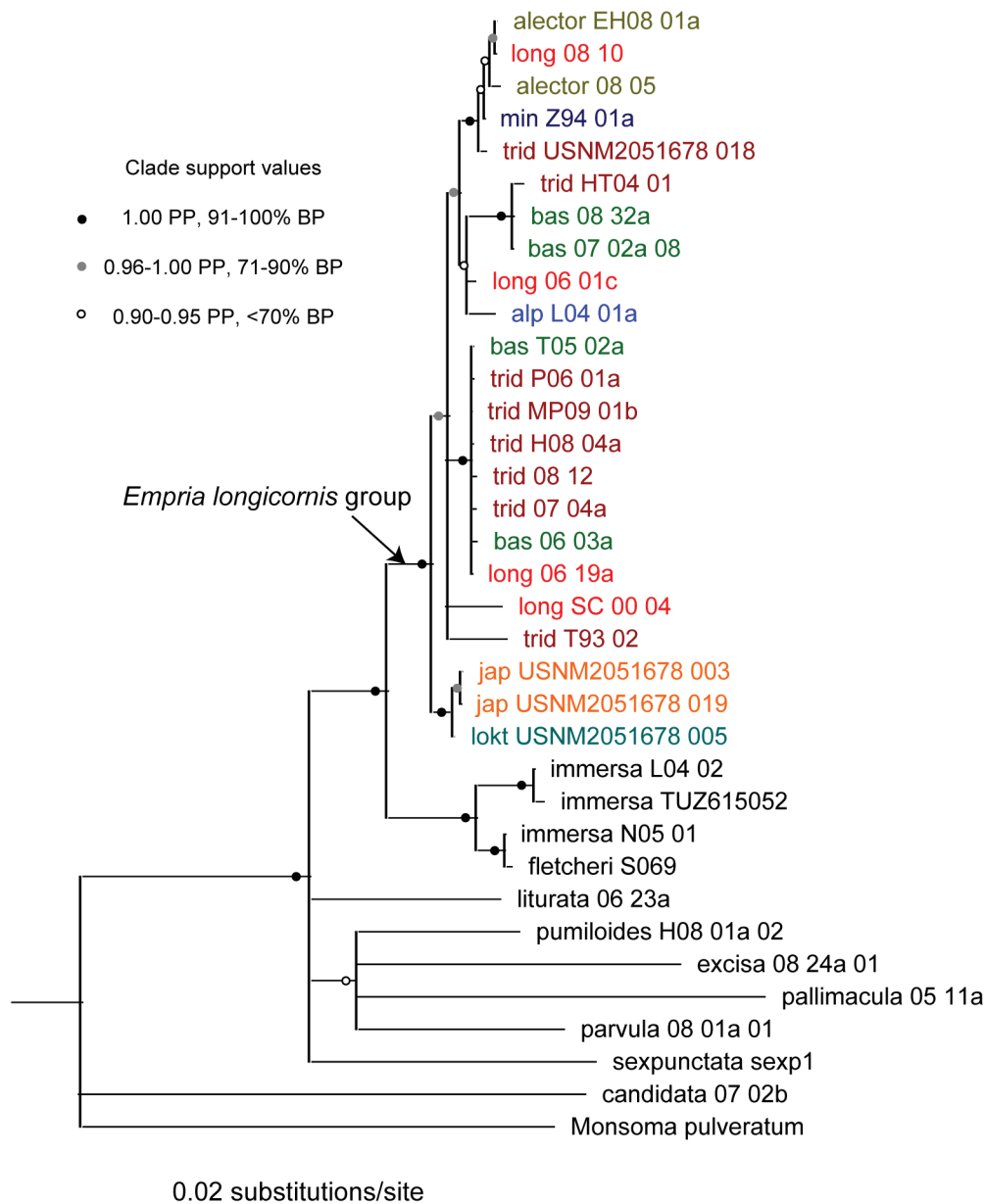


FIGURE 19. Phylogeny of mitochondrial sequences (complete COI and 3 partial tRNA) using MrBayes (GTR+4 Γ model) and including all outgroup species (alignment length 1651 bp, non-coding and ambiguously aligned tRNA regions were excluded). Clades with posterior probabilities (PP) less than 0.90 have been collapsed. Maximum likelihood (ML) analysis (GTR+4 Γ substitution model) produced nearly identical topology (few topological differences between Bayesian and ML analyses were not significantly supported, i.e. less than 0.9 PP and 0.7 BP). Bayesian and ML analyses using *E. immersa* and *E. fletcheri* as the only outgroups produced identical topologies with only slight differences in clade support values. **al**, *E. alector*; **alp**, *E. alpina*; **bas**, *E. basalis*; **jap**, *E. japonica*; **lokt**, *E. loktini*; **long**, *E. longicornis*; **trid**, *E. tridens*.

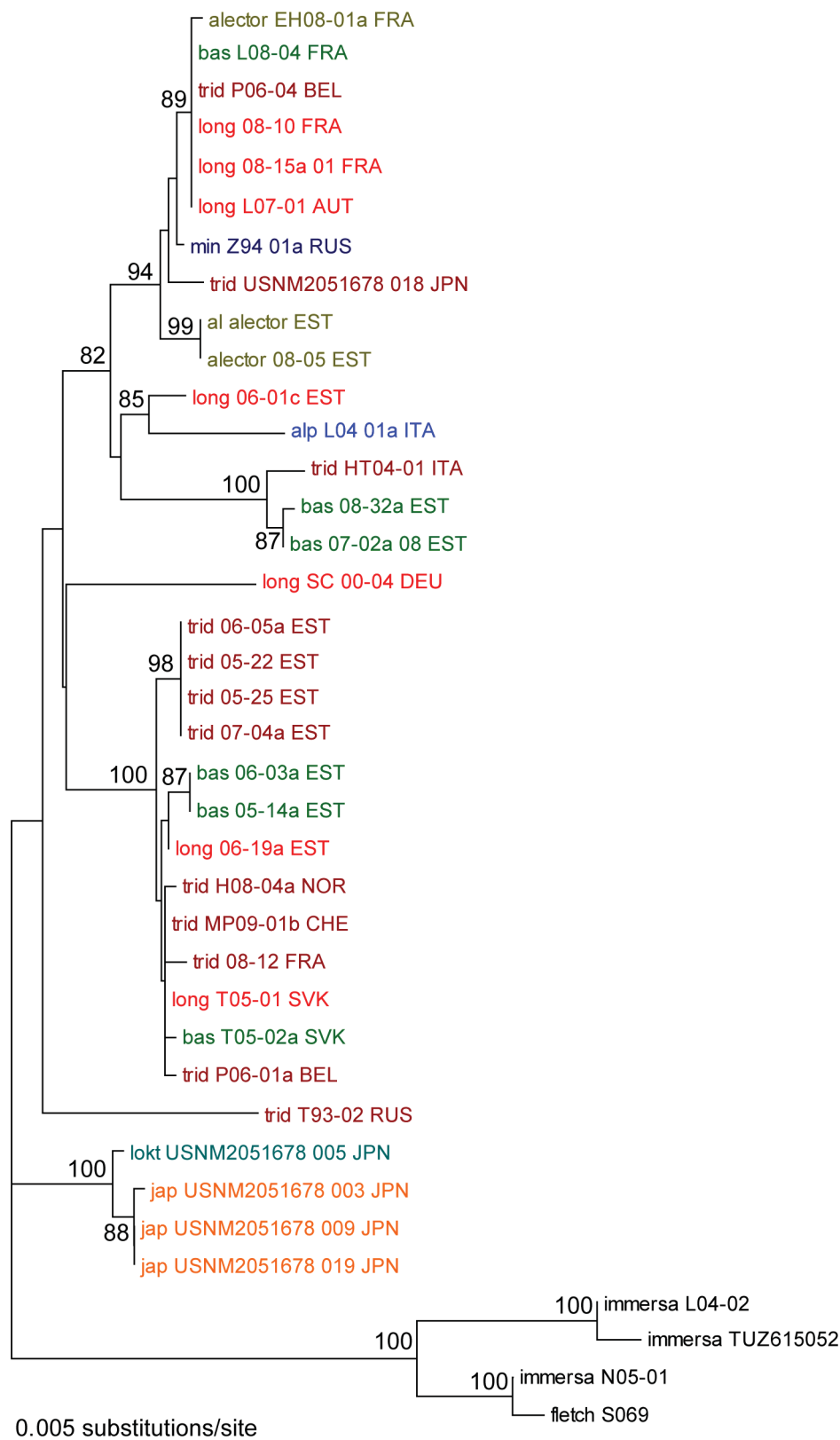


FIGURE 20. Barcoding analysis of mitochondrial sequences (complete COI, 2 partial tRNA, 1 complete tRNA, and non-coding regions) using only the closest outgroup (alignment length 1695 bp). NJ tree based on Kimura two-parameter distances, pairwise deletion. Bootstrap proportions below 70% are not shown. AUT, Austria; BEL, Belgium; CHE, Switzerland; DEU, Germany; Est, Estonia; FRA, France; ITA, Italy; JPN, Japan; NOR, Norway; RUS, Russian Federation; SVK, Slovakia; SWE, Sweden. **al**, *E. alector*; **alp**, *E. alpina*; **bas**, *E. basalis*; **fletch**, *E. fletcheri*; **jap**, *E. japonica*; **lokt**, *E. loktini*; **long**, *E. longicornis*; **trid**, *E. tridens*.

TABLE 4. Pairwise distances (number of base substitutions per site corrected with Jukes-Cantor method) of full COI (1536 bp) and ITS sequences (1303 bp) within and between *E. basalis* (n=6) and *E. longicornis* (n=7) to illustrate the conflicting signal between mitochondrial and nuclear sequences. Note that the divergence of COI sequences is similar within and between the two species, while divergence of ITS sequences is at least fourfold higher between than within species.

	<i>E. basalis</i> Average (min-max)	<i>E. longicornis</i> average (min-max)	<i>E. basalis/E. longicornis</i> average (min-max)
COI	0.013 (0.000–0.020)	0.011 (0.000–0.021)	0.012 (0.000–0.023)
ITS	0.001 (0.000–0.002)	0.001 (0.000–0.002)	0.009 (0.008–0.010)

TABLE 5. Paired-sites tests using Treefinder. Alternative topologies tested were those separately reconstructed by Treefinder (GTR+4Γ model) using mitochondrial and ITS alignments (35 taxa). For different tests, p-values are shown. The different tests were the Bootstrap Probability (BP), The Expected-Likelihood Weights by Strimmer and Rambaut (ELW), Kishino and Hasegawa (KH), Shimodaira and Hasegawa (SH), the Weighted SH test (WSH), and Approximately Unbiased test (AU). The last row shows log likelihood values.

ITS alignment		Mitochondrial alignment	
ITS phylogeny	Mitochondrial phylogeny	ITS phylogeny	Mitochondrial phylogeny
ELW, BP, KH, SH, WSH	ELW, BP, KH, SH, WSH	ELW, BP, KH, SH, WSH	ELW, BP, KH, SH, WSH
1.0	<1e-5	<1e-5	1.0
AU 0.2345	AU <1e-5	AU <1e-5	AU 0.2345
-2612.481	-3036.175	-4368.141	-3502.508

TABLE 6. Summary of the females and males reared *ex ovo*.

Species	Females		Males	
	Nr. of specimens in total	Nr. of parents (1)	Nr. of specimens in total	Nr. of parents (1)
<i>Empria basalis</i>	13	5	21	4
<i>Empria tridens</i>	6	2	16	3
<i>Empria longicornis</i>	5	4	9 (2)	3
<i>Empria alector</i>	0	NA	6	2

(1), Number of different female parents (i.e. number of independent broods), from which the total number of imagines were reared. (2), three males were reared from a female, which itself was reared *ex larva*.

Larvae and host plants

We reared *ex ovo* the larvae and imagines of the *longicornis*-group species available for study: *Empria longicornis*, *E. tridens*, *E. alector*, and *E. basalis* (Table 6). According to our ovipositing experiments (Table 7), the host plant of *E. longicornis* and *E. tridens* is *Rubus idaeus*, *E. alector* feeds on *Filipendula ulmaria*, and *E. basalis* (host plant previously unknown) on *Geum rivale*, and possibly also on *G. urbanum* (observations of Jan Macek in Czech Republic, personal communication). In addition, the larvae of *Empria tridens* (identification confirmed by ITS sequences) were collected from a plant species of *Rubus fruticosus* complex in Switzerland in 2009. Species of this complex could be additional host plants for *Empria tridens* (also supported by observations of Jan Macek, personal communication). All these species oviposit into the main stem or the leaf stem of the host plant (Fig. 21), rarely also near the leaf nerves. This trait is different from all other Palaearctic *Empria* species (Heidema & Prous 2006; Kontuniemi 1951; Liston *et al.* 2007; Lorenz & Kraus 1957; Verzhutskii 1966, 1981). However, the Nearctic *E. maculata* (Norton, 1861) is also known to oviposit into the leaf stems of *Fragaria* spp. (Smith 1979). *Empria alpina*, on the other hand, oviposits into the underside of leaf blades of *Dryas octopetala* (Veli Vikberg, personal communication), contrary to other studied species of the *longicornis*-group. We have not systematically examined the host plant spectrum of different species in the group, but some preliminary results (Table 7) suggest that it could be rather restricted. Photographs of the reared larvae are shown in Figs 23–28. Some colour characters of last instar

feeding larvae are briefly compared between the species in Table 8; more detailed data on larval morphology of *E. longicornis* group will be published with other *Empria* larvae elsewhere. We studied also three *Empria alpina* larvae (ZMH) collected from *Dryas octopetala* (Fig. 22).

TABLE 7. Results of ovipositing experiments. Column headings refer to plants and row headings to the species of *E. longicornis* group which were used in ovipositing experiments. Females used in the experiments originate from France (*E. longicornis* 08-15a and its progeny) and Estonia (all other females). Total number of experiments (1st number) and the number of experiments with the outcome of successfully reared larvae (2nd number), prepupae (3rd number), and imagines (4th number) are shown. Fu, *Filipendula ulmaria*; Fv, *Fragaria vesca*; Gr, *Geum rivale*; Ri, *Rubus idaeus*; NA, no experiments conducted.

	Ri	Fu	Gr	Fv	Successful rearings
<i>Empria longicornis</i>	6/6/5/5	1/0/0/0	1/0/0/0	1/0/0/0 (1)	6/6/5/5 (Ri)
<i>Empria tridens</i>	3/3/3/3	2/0/0/0	4/0/0/0 (2)	NA	3/3/3/3 (Ri)
<i>Empria alector</i>	1/0/0/0	2/2/2/2	NA	NA	2/2/2/2 (Fu)
<i>Empria basalis</i>	3/0/0/0 (3)	4/0/0/0 (3)	7/7/7/5	NA	7/7/7/5 (Gr)

(1), a female (reared *ex ovo* from the specimen 08-15a) laid at least one egg in the stem of the plant, but no larvae emerged.

(2), in one case, the female died without laying any eggs (no other plant species were offered for ovipositing).

(3), in one case, *Filipendula ulmaria* and *Rubus idaeus* were simultaneously available, but the female died without laying any eggs (no other plant species were offered for ovipositing).

TABLE 8. Colour characters of fully grown feeding larvae and prepupae of the species of *Empria longicornis* group.

Species	Supra-spiracular flecks	Occipital fleck	Parietal flecks	Suranal fleck	Colour of prepupa (2)
<i>Empria basalis</i>	-	+	+/-	-	gray
<i>Empria tridens</i> “type” 1 (07-04a, MP09-01b)	- (1)	+	+	+	gray
<i>Empria tridens</i> “type” 2 (06-05a, 06-06a)	-	+/-	-	-	gray
<i>Empria longicornis</i>	-	+/-	-	+	dark brownish-gray
<i>Empria alector</i>	-	+	+	+	dark brownish-gray (3)
<i>Empria alpina</i>	+	+	-	- (?)	?

(1), supraspiracular flecks can rarely be present (see Fig. 24).

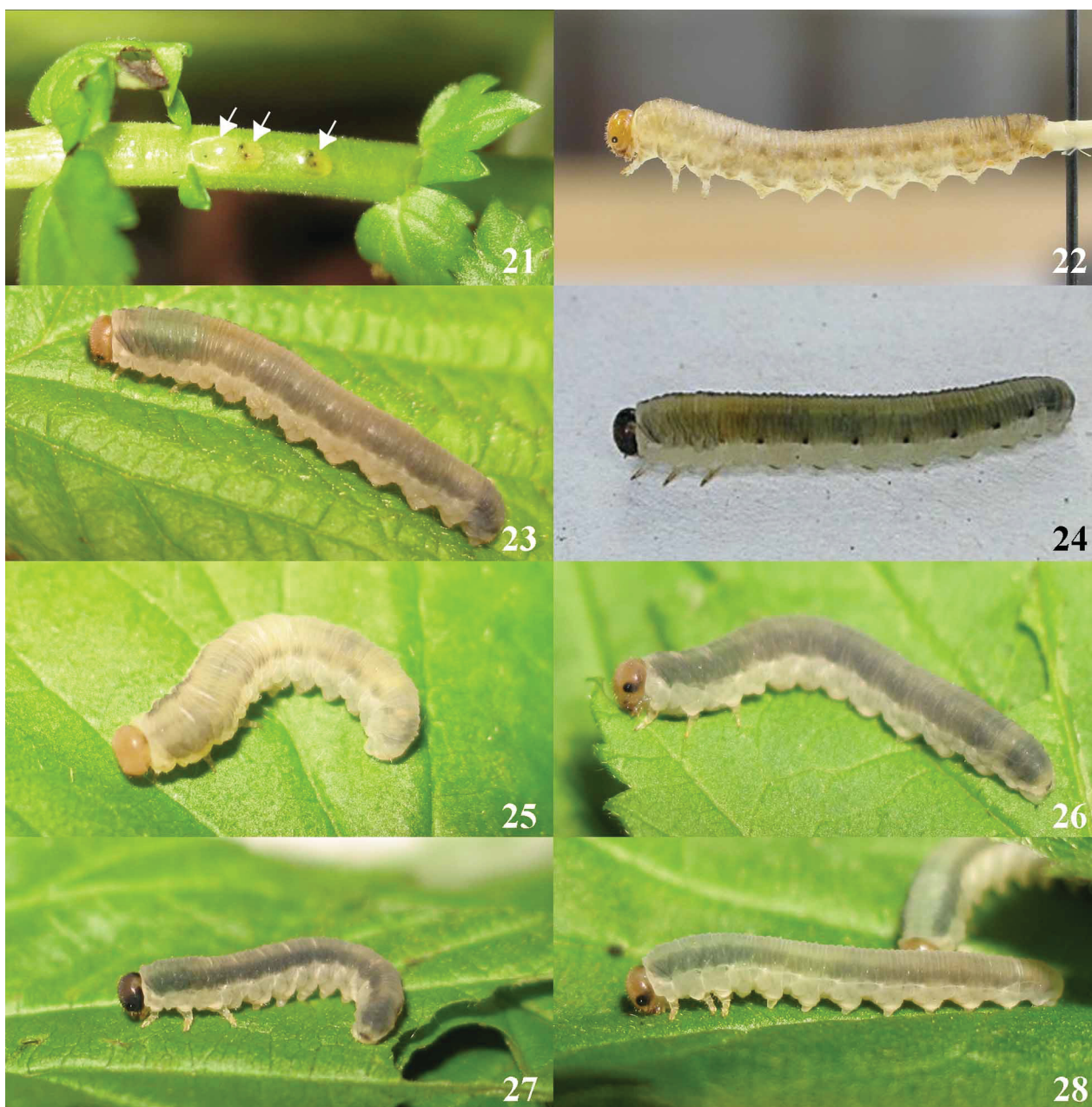
(2), see images in Morphbank: id=579010, 579012, 579014, 579016, 579018, 579021–579022, 579025–579026, 579029–579032, 579034.

(3), sometimes gray (Morphbank image id=579029–579030).

Taxonomy

Empria longicornis group is defined here using the combination of penis valve characters, which is unique within *Empria* (Fig. 9): dorsal margin of valviceps convex with minute dorso-apical tooth (often very inconspicuous) and row of teeth; apical tip of valviceps pointed and bent ventrally; and angle between dorsal and basal margin of valviceps acute, forming a notch (rarely almost absent) between valviceps and valvura. Monophyly of the *longicornis*-group is strongly supported by the analyses of DNA sequence data (Figs 16, 19). The group includes nine species at present: *E. alector*, *E. alpina*, *E. basalis*, *E. japonica*, *E. loktini*, *E. longicornis*, *E. minuta*, *E. mongolica*, and *E. tridens*.

Descriptions of new sawfly species should be accompanied by adequate illustrations of their penis valves and/or ovipositors (preferably photos). This is especially important for those species that are highly similar to some previously recorded species (as has been the case for many in the genus *Empria*). If publishing of good quality photos is problematic, these should be made accessible on the WWW (e.g. <http://www.morphbank.net/>).



FIGURES 21–28. *Empria basalis*: 21, three developing eggs (white arrows) in the stem of *Geum rivale*; 28, last instar larva. *Empria alpina*: 22, inflated larva at ZMH. *Empria longicornis*: 23, last instar larva. *Empria tridens*: 24, a larva collected (29.VI 2006, Czech Republic) and reared to adult by Jan Macek from *Rubus fruticosus* among about hundred other *E. tridens* larvae which did not have supraspiracular flecks (image courtesy of Jan Macek); 25, last instar larva (06-05a); 26, last instar larva (07-04a). *Empria alector*: 27, last instar larva.

***Empria alector* Benson, 1938**

Empria alector Benson, 1938: 191–192. Type locality: Scotland, Moray, Grantown [UK]. Holotype ♀, BMNH [not examined, see Notes].

Empria waldstaetterense Liston, 1980: 225–227. Type locality: Altdalen-St.Joder, Nidwalden, Switzerland. Holotype ♀, RSME [examined, see Notes], **syn. nov.**

Host plants. *Filipendula ulmaria*.

Distribution. West-Palaeartic. The verified country records are: Denmark, Estonia, Finland, France, Germany, Great Britain, Russia (Leningrad Oblast), Sweden, and Switzerland (complete record in the file http://empriini.myspecies.info/files/imports/long_group_spec.txt).

Key characters. Posterior margin of pronotum and paired patches on abdominal terga 2–5 (6) unpigmented; tegulae and metatibia in basal 1/3 black, sometimes partly unpigmented. Ratio of flagellum length to head breadth in females 1.8–2.1, in males mostly 2.8–3.3, and eye length to head length 1.6–2.0. Number of serrulae (16) 17–18, with its ventral margins almost at the same level along its entire length (Fig. 43). Valviceps with medium sized lobe, forming a notch between valviceps and valvura (Figs 51, 56); valvura to valviceps length ratio mostly 0.6–0.7.

Notes. Taxonomic identity is evident from the original description (e.g. fig 7c in Benson, 1938). *Empria waldstaetterense* Liston, 1980 (see Morphbank images id=579042–579045) was incorrectly synonymized with *E. sexpunctata* (Serville, 1823) by Liston (1995), under the name *E. klugii* (Stephens, 1835) at that time.

Empria alpina Benson, 1938

Empria alpina Benson, 1938: 190–191. Type locality: Scotland, Perthshire, Breadalbane Mountains, Craggs above Lochan à Lairige [UK]. Holotype ♀, BMNH [not examined, see Notes].

Host plants. *Dryas octopetala* (Veli Vikberg, personal communication).

Distribution. Holarctic (no Nearctic specimens were studied). The verified country records are: Finland, Germany, Great Britain, Italy, and Switzerland.

Key characters. Paired patches on abdominal terga 2–6 (7) unpigmented; tegulae, posterior margin of pronotum, and metatibia mostly black. Ratio of flagellum length to head breadth in females 1.6–2.1, in males 2.7–3.5, and eye length to head length 1.3–1.5. Number of serrulae 14, conspicuously papilliform (Fig. 36). Valviceps with a long basal lobe, forming very deep notch between valviceps and valvura (Fig. 53).

Notes. Taxonomic identity is evident from the original description (e.g. fig 7a in Benson, 1938), but confirmed also by studying three paratypes (1 ♀ and 1 ♂ with same collecting data as holotype).

Empria basalis Lindqvist, 1968

Empria basalis Lindqvist, 1968: 26–27. Type locality: Kallvik [from label], vicinity of Helsinki "Umgebung von Helsingfors" [original description]. Holotype ♀, ZMH [examined].

Host plants. *Geum rivale* and possibly *G. urbanum*.

Distribution. West-Palaearctic. The verified country records are: Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Russia (Leningrad Oblast and Ulyanovsk Oblast), Slovakia, Sweden, Ukraine.

Key characters. Posterior margin of pronotum and paired patches on abdominal terga 2–5 or 2–6 unpigmented; tegulae and metatibia in basal 1/3 black or partly unpigmented. Ratio of flagellum length to head breadth in females mostly 1.7–2.0, in males 2.1–2.7, and eye length to head length mostly 1.6–2.2. Number of serrulae 15–16 (rarely 14 or 17), from which 4–5 basal ones without denticles; triangular in shape (Fig. 44). Valviceps with medium sized lobe, forming a notch between valviceps and valvura (Fig. 52).

Notes. The paratype male (labelled as allotype) was also studied.

Empria japonica Heidemaa & Prous, sp. nov.

urn:lsid:zoobank.org:act:BA25596E-802D-43E3-B351-52A0BAB1B78F

Holotype, 1 ♀: "JAPAN, Hokkaido Ginsendai, Kamikawa-chô 43°40'N, 143°01'E, 947 m selectively cut forest 6–27.vi.2008 Mal. trap, A. Ueda leg" [white, printed]; "USNM2051678 019" [specimen ID; white, printed and handwritten]; "HOLOTYPUS ♀ *Empria japonica* spec. nov. 2010, design. M. Heidemaa & M. Prous" [red, printed]; "*Empria japonica* Heidemaa & Prous det. 2010" [white, printed]; valvula 1 is mounted in euparal between rectangular cover slips and pinned with the specimen (NSMT).

Paratypes: "JAPAN, Hokkaido Ginsendai, Kamikawa-chô 43°40'N, 143°01'E, 947 m selectively cut forest 6–27.vi.2008 Mal. trap, A. Ueda leg" [white, printed], 1 ♀, 2 ♂ (USNM), 1 ♀ (SDEI); "JAPAN, Hokkaido Ginsendai,

Kamikawa-chô 43°40'N, 143°01'E, 947 m selectively cut forest 27.vi.–18.vii.2008 Mal. trap, A. Ueda leg" [white, printed], 1 ♂ (USNM); "JAPAN, Hokkaido Sekihoku-tôge, Kamikawa-chô, natural forest, 993 m 43°40'N, 143°06'E, 6–27.vi.2008 Mal. trap, A. Ueda leg" [white, printed], 5 ♀, 1 ♂ (USNM), 1 ♀ 1 ♂ (TUZ), 1 ♂ (SDEI); "JAPAN, Hokkaido Uenzaru-gawa, Hidaka-cho 42°55'N, 142°45'E, 1160 m natural forest 10.vii.–1.viii.2008 Mal. trap, A. Ueda leg" [white, printed], 1 ♀ (USNM); "[JAPAN Hokkaido] Akadake Ginsendai, Daisetsuzan Mts. 23. VI. 2005 Mal. trap H. Hara & A. Shinohara" [white, printed], 1 ♂ (NSMT); "[JAPAN:Hokkaido] Asahidake-onsen, Daisetsuzan Mts. 43-38-50N 142-47-27E 1050m 23–26. VI. 2007 A. Shinohara" [white, printed], 1 ♀ (NSMT); "[JAPAN:Hokkaido] Horoshika-toge 1100m, Tokachi 21–25. VI. 1997 A. Shinohara" [white, printed], 1 ♂ (NSMT); "[JAPAN:Hokkaido] Yamada-onsen 800m, Tokachi 21–24. VI. 1997 A. Shinohara" [white, printed], 3 ♂ (NSMT); "[JAPAN:Hokkaido] Yamada-onsen 1000m, Tokachi 21–24. VI. 1997 A. Shinohara" [white, printed], 5 ♂ (NSMT); "[JAPAN:Hokkaido] Yamada-onsen 800–1000m, Tokachi 19. VI. 1998 A. Shinohara" [white, printed], 1 ♂ (NSMT).

Female. Length 6.4–7.1 mm (AVR=6.56 SD=0.25; n=7).

Male. Length 5.8–6.7 mm (AVR=6.20 SD=0.39; n=8).

Colour. Black; following parts unpigmented, pale: labrum, apical segments of maxillar and labial palps, tegulae, posterior margin of pronotum, femora apically, protibia and mesotibia in anterior and posterior aspects (dorsal and ventral aspects pigmented at least partially), metatibia in basal 1/3–1/2, basal tarsomeres at least partially, paired patches on abdominal terga 2–6 (♀, ♂) or 2–5 (♂), posterior margins of terga and sterna (very narrowly), and cenchri; harpes sometimes at least partially unpigmented at margins.

Head: from parallel sided to subparallel sided behind eyes in dorsal view. Postocellar field trapeziform with postocellar furrows diverging backwards, slightly longer than the length of ocellar area; area between frontal crests barely reaches the level of crests (Fig. 4) or at most exceeds this level very little in dorsal view (head positioned so posterior margins of lateral ocelli and compound eyes aligned, Fig. 2). Distinct punctures on upper head absent, postocellar area glossy, showing more or less wrinkled sculpture on postocular area and between compound eyes. Face and particularly clypeus with irregular sculpture, with fused punctures dominant on clypeus. Ocellar area and postocellar area slightly raised. Clypeus tridentate with raised median tooth smaller than lateral teeth. Minimal ventro-ocular distance in females slightly shorter than distance between antennal sockets (ratio 0.75–0.9), in males about equal or slightly shorter (0.85–1.0). Frontal ridge "V"-shaped, disrupted before reaching the level of middle ocellus, frontal field resembles triangular depression, often with a pit in its central part (between ridges), but raising, sometimes convex in front of middle ocellus. Maximal temple length (maximal distance between compound eye and occipital carina at the central portion of eye - TL max) about 1.45–1.55 times exceeds minimal temple length (minimal distance between compound eye and occipital carina at the lower portion of eye - TL min) in lateral view (Fig. 3). The ratio of eye length to length of head behind the compound eyes 1.4–1.6. Postocellar region in males 1.8–2.0 times and in females about 2.5 as long as the ocellar diameter. Temples at genal orbits in the middle part of eyes with a longitudinal flat region (Fig. 3), sometimes more or less depressed near compound eye (usually more distinctly in specimens dried from alcohol). Antennae slender, in female almost as long as the distance from tegula to the mid of pterostigma, in males even longer, reaching the apex of pterostigma. Ratio of flagellum length and head breadth in females 2.5–2.7 (n=4), in males 3.2–3.8 (n=6).

Thorax: median mesoscutal lobes with inner and lateral regions of the lateral mesoscutal lobes impunctate and glossy, the median regions of lateral mesoscutal lobes with shallow puncture and glossy interspaces. Mesoscutellum and mesoscutellar appendage impunctate, glossy. Axillae smooth or with barely visible sculpticells in lower portion, glossy. Distance between cenchri about equal to cenchrus width. Metapostnotum impunctate and glossy, sometimes showing wrinkled sculpture in its central part (behind metascutellum). Metascutellum impunctate and rather glossy but can have some shallow transversal wrinkles (not sculpticell like) at least in anterior part, posterior part with similar or more uneven sculpture. Mesepisternum in lateral region glossy with minute punctures only around setae in upper part, pectal region with larger and more irregular puncture but glossy interspaces. Metepisternum with evenly distributed setae, metepimeron in central part without setae. Mesepimeron with setae on posterior part only. Wings from hyaline to very lightly infuscated; venation brownish (see Morphbank image id=579063), paler at the base, some basal sclerites can be partially unpigmented, whitish. Tarsal claw with a subbasal tooth reaching about halfway between its base and the apex of claw (shorter in males).

Abdomen: terga with scale- and keel-like sculpticells and short setae (about half of the ocellar diameter) at least on medio-anterior and lateral parts of terga. Valvula 3 on lateral surface with keel-like and scale-like sculpti-

cells, gradually narrowing towards its apex in dorsal view with curved setae mostly shorter than ocellus diameter. Valvifer 2 in posterior portion with irregular chain-like rows of punctures which can be partly fused. Ventral margins of valvula 3 and valvifer 2 about equal length. Valvula 1 as in Fig. 40, number of serrulae 16–17. In males posterior edge of sternum 9 straight. Genital capsule in dorsal and ventral view as in Figs 5–6, valviceps of penis valves with short lobe, forming shallow notch between valviceps and valvura (Fig. 47); valvura to valviceps length ratio 0.58–0.62.

Taxonomic affinities. The newly described species resembles *E. longicornis* Thomson habitually by its fairly slender body and very long antennae. However, in *E. japonica* the temple length ratio (TL max / TL min) is mostly between 1.45–1.55 (Fig. 3), in other species, except in *E. alpina* (the ratio is around 1.5), it is mostly between 1.2–1.35 (Fig. 7). Frontal ridge is not disrupted in *E. longicornis* before reaching the level of middle ocellus, like in *E. japonica*. Unlike in *E. japonica*, the frontal field in *E. longicornis* forms a longitudinal depression almost reaching the middle ocellus. Area between frontal crests in dorsal view is usually much more prominent in *E. longicornis*, usually clearly exceeding the level of crests (Fig. 8). Males resemble most those of *E. alpina*, but the males of *E. japonica* have the frontal ridge disrupted, non-glossy frontal area with wrinkles, and differently shaped penis valves. Serrulae of the species are most similar to *E. tridens*, but the absolute as well as relative length of the basal-most annulus of valvula 1 (see Fig. 10) is longer (absolute length exceeds 0.1 mm, relative length exceeds 0.065; n=3) compared to *E. tridens* (n=18) and *E. longicornis* (n=14). Relative height of valvula 1 (see Fig. 10) is also different (exceeds 0.115) from *E. tridens* (less than 0.108), but not from *E. longicornis*. The penis valve is most similar to *E. tridens*.

Host plants. Unknown, but could be *Rubus idaeus* ssp. *melanolasius*, which is common in the localities where at least some of the *E. japonica* specimens have been collected (A. Shinohara, personal communication).

Distribution. Japan (Hokkaido).

Empria loktini Ermolenko, 1971

Empria loktini Ermolenko, 1971: 22–23. Type locality: Novoaleksandrovsk [Sakhalin Oblast, Russia]. Holotype ♀, (SIZ) [examined].

Host plants. Unknown, but the adults have been collected from *Filipendula kamtschatica* (see Ermolenko 1971) and at least some of the Japanese specimens have been collected from the localities where this plant species is common (A. Shinohara, personal communication).

Distribution. East-Palaearctic. The verified country records are: Japan (Hokkaido) and Russia (Sakhalin Oblast).

Key characters. Posterior margin of pronotum, metatibia in basal 1/3, and paired patches on abdominal terga 2–3 or 2–4 (5) unpigmented; tegulae unpigmented (females) or black (males). Ratio of flagellum length to head breadth in females 1.8–1.9, in males 2.3–2.4, and eye length to head length in females 1.2–1.7, in males 1.5–2.0. Number of serrulae 13–14 (15), triangular in shape (Fig. 39). Penis valve with relatively large apical tooth and apical part of valvular duct going clearly further from dorsal rim of valvura; valviceps with medium sized lobe, forming a notch between valviceps and valvura (Fig. 55).

Notes. The paratypes 1 ♀ 2 ♂ (1 ♂ labelled as allotype) were also studied.

Empria longicornis (Thomson, 1871)

Poecilosoma longicornis Thomson, 1871: 232. Type locality [original description]: Skåne [Southern Sweden]. Lectotype (**here designated**) ♀: “ZML 2006 362” [pale, printed]; “LECTOTYPUS 2009 *Poecilosoma longicornis* THOMSON, 1871 M.Heidema & M.Prous design.” [red, printed]; “*Empria longicornis* (Thomson, 1871) det. M.Prous 2007” [white, printed]; paralectotypes (representing different *Empria* spp.): 8 ♀ and 2 ♂; all ZML.

Empria rubi Kontuniemi, 1951: 24–27. Type locality [from label]: Pernaja, Suomi U. [Finland, “U.” refers to Uusimaa District]. Holotype ♀, ZMH [examined, see Notes].

Host plants. *Rubus idaeus*. *Filipendula ulmaria* (see Kontuniemi 1951) and *Fragaria vesca* (see Zhelochovtsev & Zinovjev 1988) were not confirmed as preferred host plants for *E. longicornis* (Table 7).

Distribution. Palaearctic. The verified country records are: Denmark, Estonia, Finland, France, Germany, Hungary, Romania, Slovakia, Sweden, and Switzerland. The record from Mongolia (Zombori 1972) is incorrect due to misidentification of several *E. mongolica* males as *E. longicornis*.

Key characters. Posterior margin of pronotum and paired patches on abdominal terga 2–4 or 2–5 (6) (mostly males) unpigmented; tegulae and metatibia in basal 1/3 black, sometimes partly unpigmented. Ratio of flagellum length to head breadth in females mostly 2.3–2.7, in males mostly 3.1–3.8, and eye length to head length 1.7–2.2. Number of serrulae 16–17 (18), triangular in shape (Figs 41, 46). Valviceps with short lobe, forming inconspicuous notch between valviceps and valvura (Fig. 48); valvura to valviceps length ratio mostly 0.45–0.56.

Notes. The type specimens of *Empria rubi* Kontuniemi, 1951 (2 ♀, 2 ♂) were reared from larvae (holotype ♀, 2 paratypes ♀ and ♂ [labelled as allotype]) or *ex ovo* (paratype ♂) by T. Kontuniemi.

Empria minuta Lindqvist, 1968

Empria minuta Lindqvist, 1968: 30. Type locality: vicinity of Helsinki [original description] “Umgegend von Helsingfors” [Finland], “N. Helsing” [from label]. Holotype ♀, ZMH [examined].

Empria gussakovskii auct. nec Dovnar-Zapolskij, 1929.

Host plants. Unknown.

Distribution. Palaearctic. The verified country records are: Estonia, Finland, Mongolia, Russia (Leningrad Oblast, Yamalo-Nenets Autonomous Okrug), and possibly Sweden (the specimen UUZM_HS).

Key characters. Tegulae partly or largely, posterior margin of pronotum, metatibia in basal 1/3, and paired patches on abdominal terga 2–6 unpigmented. Ratio of flagellum length to head breadth in females 1.8–2.1, in males 2.8–3.0, and eye length to head length in females 1.6–1.7, in males 1.7–2.1. Number of serrulae 14–16, slightly papilliform shaped (Fig. 38). Valviceps with medium sized lobe, forming a notch between valviceps and valvura (Fig. 58); valvura to valviceps length ratio 0.63–0.72.

Notes. There is no information about the locality and the collector of the female specimen from UUZM, but it has probably been collected from Sweden. The flight period of the adults might partly explain why this species has been collected rarely. The Estonian specimens were found in a Malaise trap only between 12–30 April (3 ♂) and 30 April – 15 May 2010 (1 ♀).

Empria mongolica (Konow, 1895)

Poecilosoma mongolica Konow, 1895: 75. Type locality: northern Mongolia “Mongolia bor[ealis]”. Lectotype (**here designated**) ♀: “N. Mongolei Leder 92” [white, printed]; “*Poecilosoma Mongolica* Knw. Mongol. bor.” [white, framed, handwritten]; “Typus” [red, printed]; “Holotypus” (Sic!) [red, printed]; “Coll. Konow” [white, printed]; “Conde revid. 1937” [white, handwritten]; “GBIF-GISHym 3782” [white, printed]; “**LECTOTYPUS** *Poecilosoma mongolica* KONOW, 1895 ♀ M.Heidemaa & M.Prous des.” [red, printed]; “*Empria mongolica* (Konow, 1895) det. M.Prous 2008” [white, printed]; SDEI (see Notes).

Host plants. *Dasiphora fruticosa* (see Verzhutskii 1981).

Distribution. East-Palaearctic. The verified country records are: Mongolia, Russia (Kamchatka Krai, Irkutsk Oblast: Verzhutskii 1981). The record from arctic Norway (Kiaer 1898) is incorrect due to original misidentification of *E. fletcheri* specimens (determined by E. Lindqvist in 1948) as *E. mongolica*.

Key characters. Posterior margin of pronotum, small basal part or basal 1/3 of metatibia, and paired patches on abdominal terga 2–6 (7) unpigmented; tegulae largely unpigmented (females) or black (males). Ratio of flagellum length to head breadth in females 1.7–2.0, in males 2.7–3.2, and eye length to head length in females 1.5–1.7, in males 1.7–2.2. Number of serrulae 14–15, conspicuously papilliform shaped (Fig. 37). Valviceps with a long basal lobe, forming relatively deep notch between valviceps and valvura (Fig. 54).

Notes. Oehlke & Wudowenz (1984) assumed that the only female of *Poecilosoma mongolica* Konow, 1895 found in Konow’s collection is the holotype but neither holotype was designated nor explicitly stated in the original description that there was only one specimen (thus a syntype).

Empria tridens (Konow, 1896)

Poecilosoma (*Poecilosoma*) *tridens* Konow, 1896: 54, 58. Type locality: Europe “*Europa fere tota*” [original description]. Lectotype (**here designated**) ♀: “Satrup. 19.5.82” [pale, handwritten: 19.V.1882 (not VIII!), possibly Satrupholz around Sønderborg, Denmark; Fig. 35]; “Coll. Konow” [white, printed]; “Syntypus” [red, printed]; “*Empria tridens* Knw ♀ O. Conde det. 1936.” [white, printed and handwritten]; “GBIF-GISHym 3792” [white, printed]; “Lectotypus, *Poecilosoma tridens* Konow, 1896 Des. M. Heidemaa & M. Prous 2009” [red, printed]; “*Empria tridens* (Konow, 1896) det. M.Prous 2008” [white, printed]; paralectotypes: 2 ♂, all SDEI.

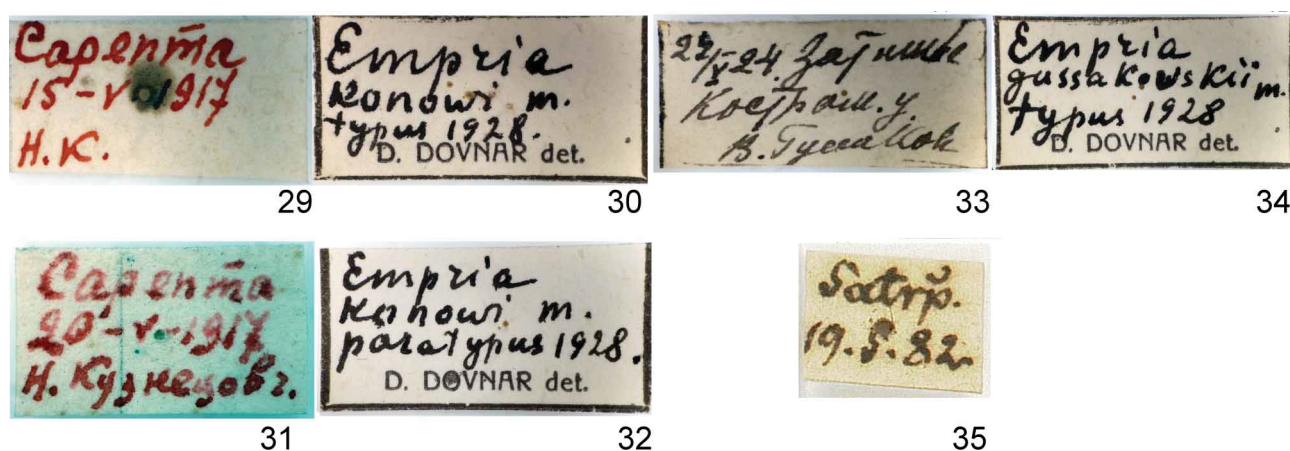
Empria (*Triempria*) *konowi* Dovnar-Zapolskij, 1929: 39–40. Type locality: Sarepta [Russia]. Lectotype (**here designated**) ♀: [original labels as in Figs 29, 30]; “**LECTOTYPUS** *Empria konowi* Dovnar-Zapolskij, 1929 ♀ M.Heidemaa & M.Prous des.” [red, printed]; “*Empria tridens* (Konow, 1896) det. M.Prous 2008” [white, printed]; paralectotype ♀ [original labels as in Figs 31, 32]; all SIZ; **syn. nov.**

Empria (*Triempria*) *gussakovskii* Dovnar-Zapolskij, 1929: 40–41. Type locality: Kostroma District [Russia], Lectotype (**here designated**) ♀: [original labels as in Figs 33, 34]; “**LECTOTYPUS** *Empria gussakovskii* Dovnar-Zapolskij, 1929 ♀ M.Heidemaa & M.Prous des.” [red, printed]; “*Empria tridens* (Konow, 1896) det. M.Prous 2008” [white, printed]; SIZ; **syn. nov.**

Empria (*Empria*) *caucasica* Dovnar-Zapolskij, 1929: 38–39. Type locality: North-West Caucasus, Kluchor, beside Teberda [original description] “Nord-West-Kaukasus, Kluchor, neben Teberda” [Karachay-Cherkess Republic, Russia]. Synonymy according to Conde (1940), see Notes.

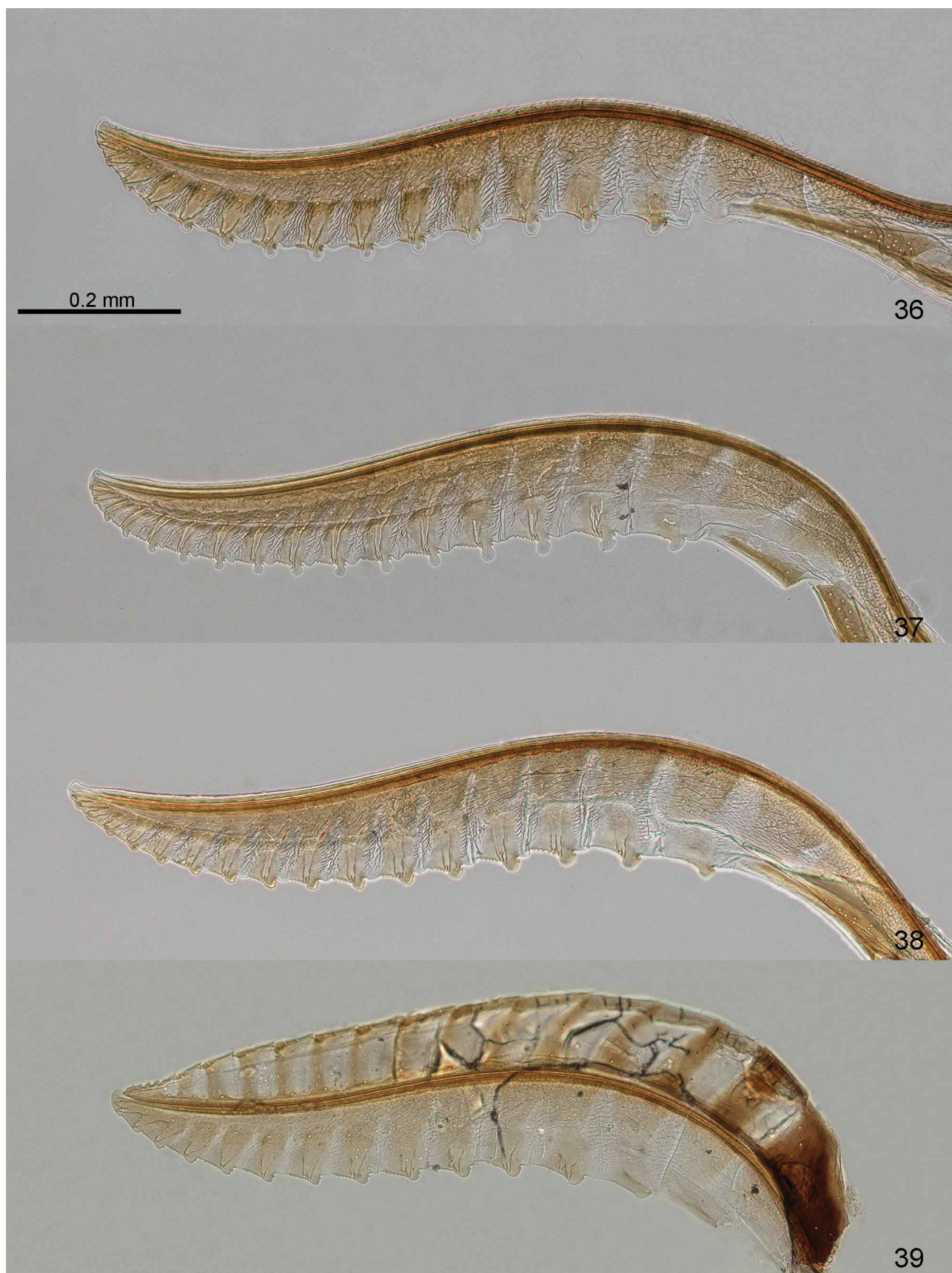
Host plants. *Rubus idaeus* and possibly *Rubus fruticosus* complex. According to Conde (1934) also *Geum*, but because no *ex ovo* rearings were done, it is possible that he observed larvae of *E. basalis*.

Distribution. Palaearctic. The verified country records are: Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Japan, Mongolia, Russia (Amur Oblast, Kamtschatka Krai, Kostroma Oblast, Leningrad Oblast, Primorskiy Kray, Sakhalin Oblast, Stavropol Krai, Volgograd Oblast), Sweden, Switzerland, Turkey, Ukraine.

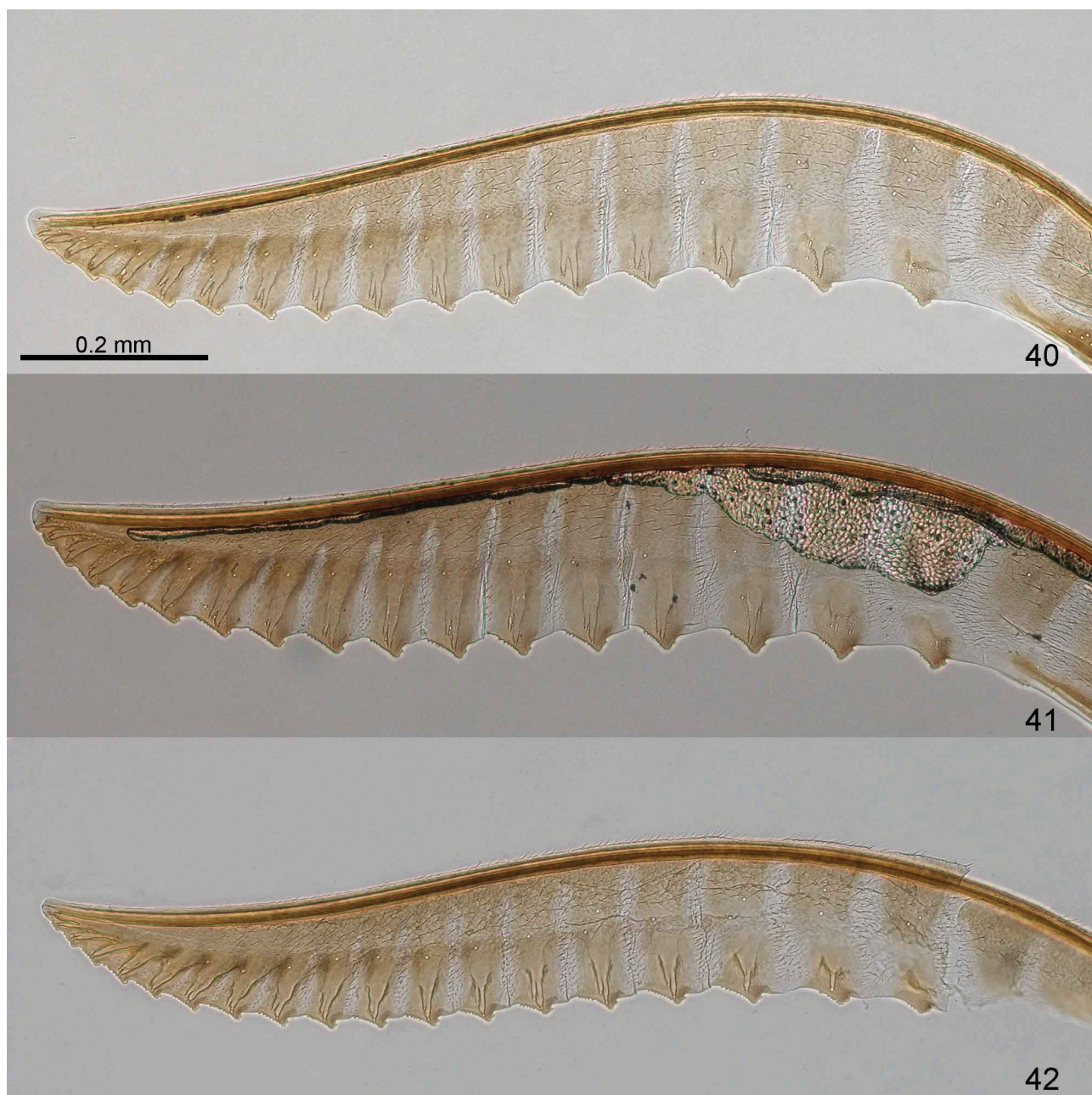


FIGURES 29–35. *Empria konowi* Dovnar-Zapolskij, 1929: 29, locality label of the lectotype; 30, determination label of the lectotype; 31, locality label of the paralectotype; 32, determination label of the paralectotype. *Empria gussakovskii* Dovnar-Zapolskij, 1929: 33, locality label of the lectotype; 34, determination label of the lectotype. *Poecilosoma tridens* Konow, 1896: 35, locality label of the *Empria tridens* (Konow, 1896) lectotype (image courtesy of SDEI).

Key characters. Posterior margin of pronotum and paired patches mostly on abdominal terga 2–6 unpigmented; tegulae and metatibia in basal 1/3 vary from unpigmented to black. Ratio of flagellum length to head breadth in females mostly 1.9–2.3, in males mostly 2.4–3.1, and eye length to head length 1.6–2.2. Number of serrulae 16–18, triangular in shape (Figs 42, 45). Valviceps with short lobe, forming inconspicuous notch between valviceps and valvura (Figs 49–50); valvura to valviceps length ratio mostly 0.52–0.63.

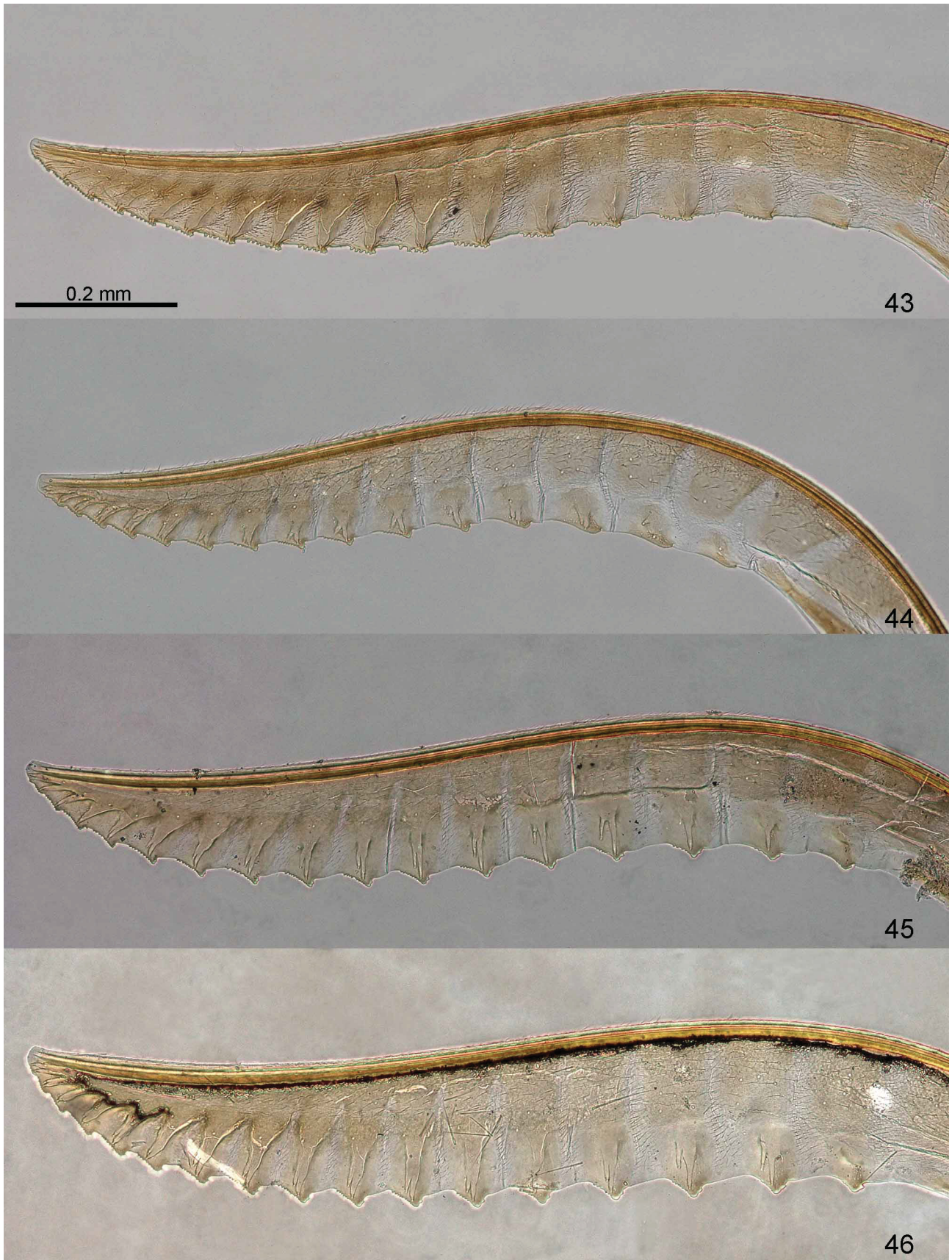


FIGURES 36–39. Lancets (valvulae 1) / lancet (valvula 1) and lance (valvula 2) of *Empria longicornis* group: 36, *E. alpina*, lancet; 37, *E. mongolica*, lancet; 38, *E. minuta*, lancet; 39, *E. loktini* (holotype), lancet and lance.

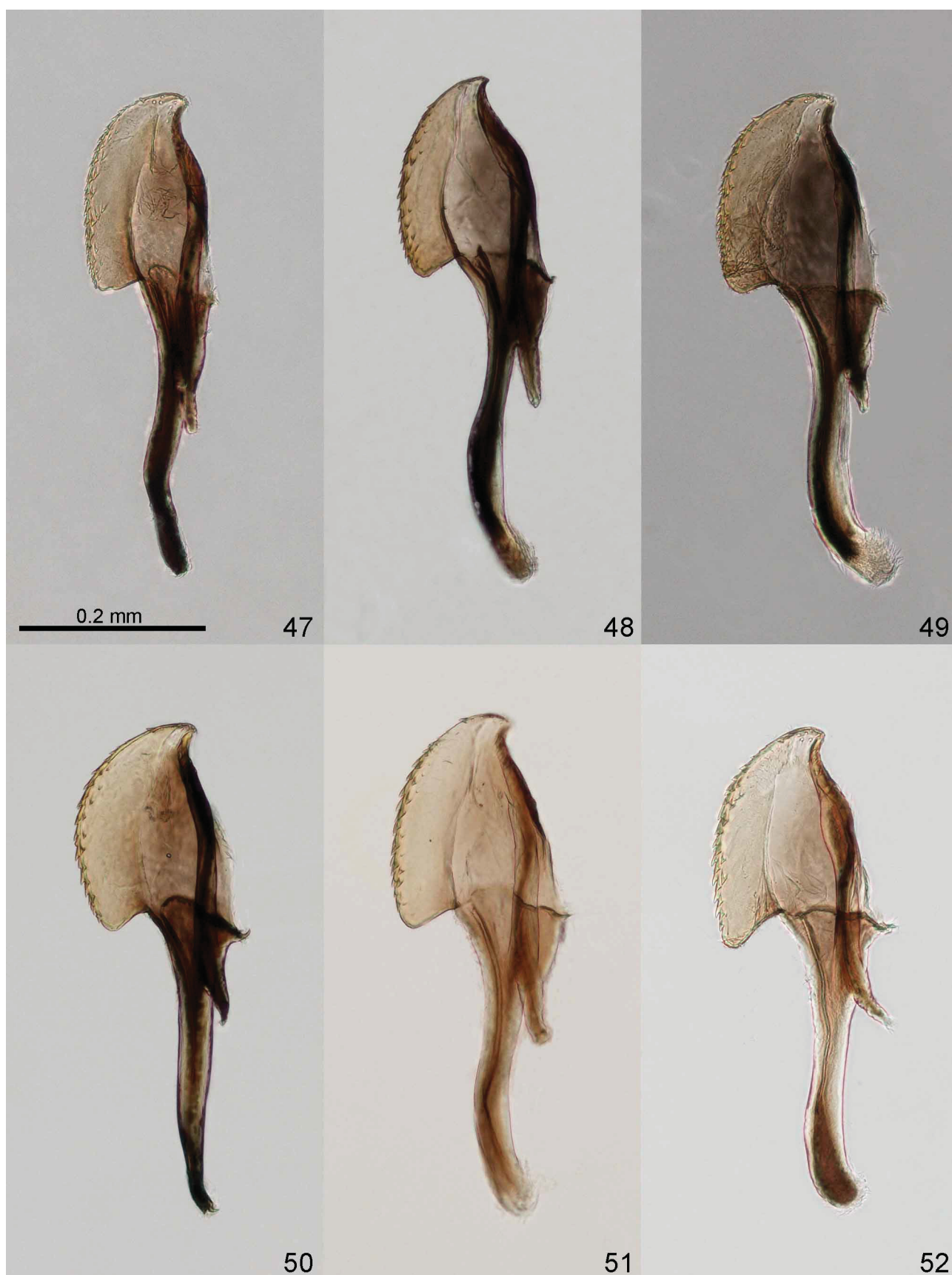


FIGURES 40–42. Lancets (valvulae 1) of *Empria longicornis* group: 40, *E. japonica*; 41, *E. longicornis*; 42, *E. tridens*.

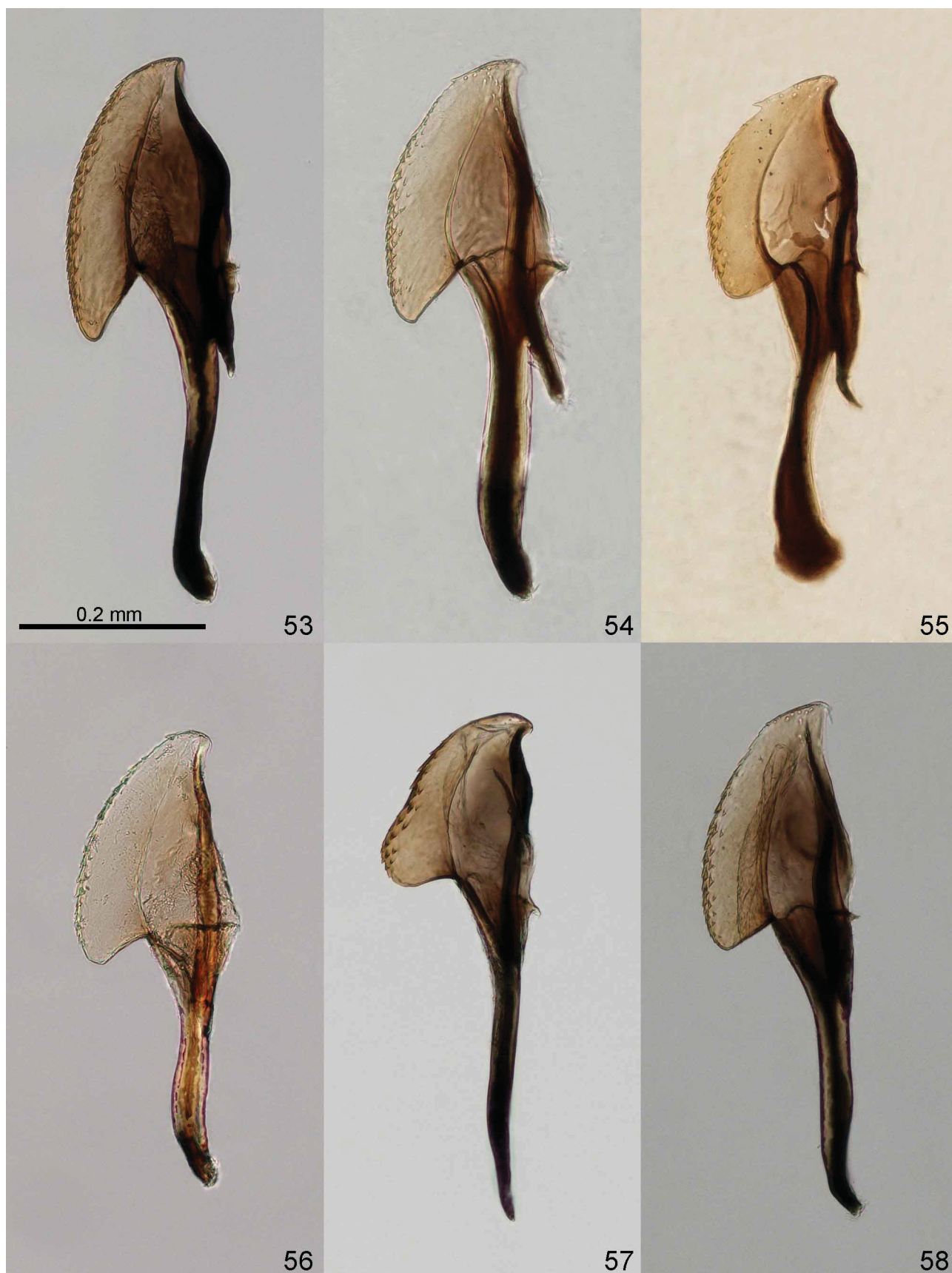
Notes. Dovnar-Zapolskij (1929) did not designate the holotype of *E. konowi* but only labelled one female as “m. [mihi] typus” (from Sarepta) and at least two females (from Sarepta and from Stavropolj) as “m. paratypus” (original labels of 2 syntype ♀ are illustrated in Figs 29–32). Though Conde (1940) stated that the type of *Empria konowi* was from Sarepta (“Die Type stammt aus Sarepta”), he examined in fact only one syntype female (“Paratype” aus Stavropolj 4.V.1921” [not examined, probably lost]). Because there were additional (syntype) females of *E. konowi* (at least the two from Sarepta) which Conde had not examined, the statement by Conde (1940) does not constitute a valid lectotype designation before 2000 (Articles 74.5, 74.6, ICZN 1999). Also, for *E. gussakovskii* Dovnar-Zapolskij, 1929 neither the holotype was fixed by the author nor explicitly indicated in the original description that there was only one specimen. No syntypes of *Empria caucasica* Dovnar-Zapolskij, 1929 were found among Dovnar-Zapolskij’s type material. Thus, we cannot exclude the possibility that it represents *E. alector* or *E. basalis*, because these two resembling species were unknown to Conde or not recognized by him (Conde 1940).



FIGURES 43–46. Lancets (valvulae 1) of *Empria longicornis* group: 43, *E. alector*; 44, *E. basalis*; 45, *E. tridens* (specimen h-02a); 46, *E. longicornis* (specimen h-22a).



FIGURES 47–52. Penis valves of *Empria longicornis* group: 47, *E. japonica*; 48, *E. longicornis*; 49–50, *E. tridens*; 51, *E. alector*; 52, *E. basalis*.



FIGURES 53–58. Penis valves of *Empria longicornis* group: 53, *E. alpina*; 54, *E. mongolica*; 55, *E. loktini* (paratype); 56, *E. alector* (specimen h-32); 57, *E. tridens* (specimen 08-12); 58, *E. minuta*.

Key to species (imagines)

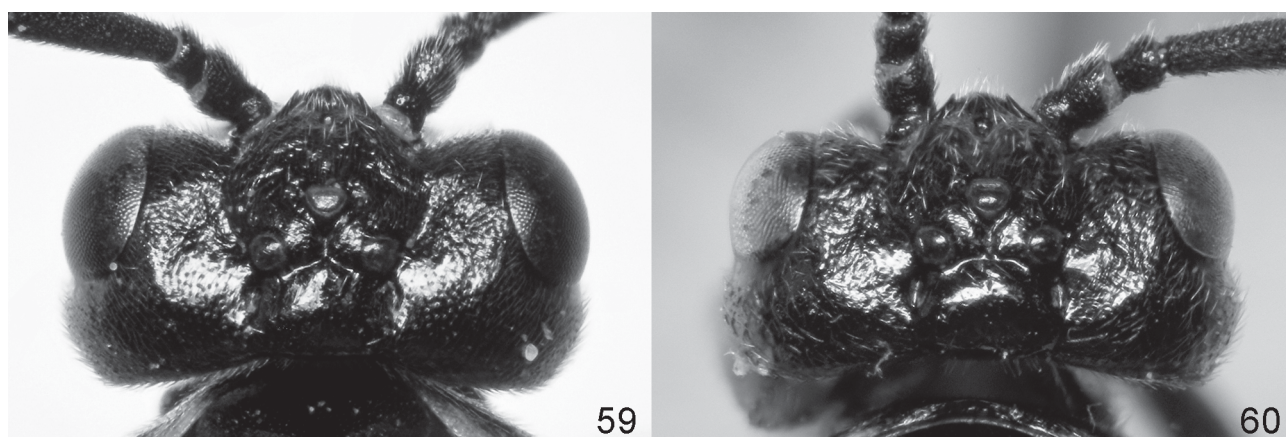
1. ♀ 2
- ♂ 10
2. Serrulae papilliform (Figs 36–38) 3
- Serrulae not papilliform (Figs 39–46) 5
3. Serrulae conspicuously papilliform (Figs 36–37) 4
- Serrulae not conspicuously papilliform (Fig. 38) and the ratio of eye length to head length exceeds 1.6 *E. minuta*
4. Serrulae as in Fig. 36; the ratio of eye length to head length less than 1.4; posterior margin of pronotum black *E. alpina*
- Serrulae as in Fig. 37; the ratio of eye length to head length exceeds 1.5; posterior margin of pronotum unpigmented *E. mongolica*
5. Serrulae with ventral margin almost at the same level along its entire length, not protruding (Fig. 43) *E. alector*
- Serrulae of triangular shape with basal part protruding (Figs 39–42, 44–46) 6
6. Serrulae weakly developed and 4–5 basal-most serrulae without denticles (Fig. 44) *E. basalis*
- Serrulae more robust and 0–3 basal-most serrulae without denticles (Figs 39–42, 45–46) 7
7. Serrulae as in Fig. 39; number of serrulae 13–14 (15) *E. loktini*
- Serrulae different, more triangular in shape (Figs 40–42, 45–46); number of serrulae exceeds 14 (mostly 16–18) 8
8. TL max exceeds TL min (Fig. 3) about 1.45–1.55 times; the area between frontal crests mostly does not exceed the level of crests in dorsal view (Fig. 4); length of the basal-most annulus of valvula 1 (Fig. 10) exceeds 0.1 mm (relative length exceeds 0.065); length of the flagellum 2.5–2.7 times of the head breadth *E. japonica*
- TL max exceeds TL min about 1.2–1.35 times (Fig. 7); the area between frontal crests mostly exceeds the level of crests in dorsal view (Fig. 8); length of the basal-most annulus of valvula 1 (Fig. 10) less than 0.1 mm (relative length less than 0.065) . 9
9. Serrulae as in Figs 41, 46; length to breadth ratio of flagellomere 3 mostly exceeds 3.5; length of the flagellum mostly 2.3–2.7 times of the head breadth; abdominal terga with 3 pairs of large and 1 pair of small whitish flecks *E. longicornis*
- Serrulae as in Figs 42, 45; length to breadth ratio of flagellomere 3 mostly less than 3.5; length of the flagellum mostly 1.9–2.3 times of the head breadth; abdominal terga with 4 pairs of large and 1 pair of small whitish flecks *E. tridens*
10. Penis valve with a deep notch (Figs 53–54) and flagellum length mostly exceeds 2.7 times of the head breadth 11
- Penis valve with a shallow notch (Figs 47–52, 55–58), the ratio of eye length to head length mostly exceeds 1.6 12
11. Penis valve as in Fig. 53; the ratio of eye length to head length about 1.3–1.5; posterior margin of pronotum black . . . *E. alpina*
- Penis valve as in Fig. 54; the ratio of eye length to head length exceeds 1.6; posterior margin of pronotum unpigmented *E. mongolica*
12. Flagellum length exceeds 2.7 times of the head breadth. (*E. alector*, *E. longicornis*, *E. minuta*, *E. tridens* in part) ... 13
- Flagellum length less than 2.7 times of the head breadth (*E. basalis*, *E. loktini*, *E. tridens* in part) ... 17
13. Basal lobe of the valviceps long (Figs 51, 56, 58), valvura to valviceps length (Fig. 9) ratio exceeds 0.57 (mostly exceeds 0.6) (*E. alector*, *E. minuta*) 14
- Basal lobe of the valviceps short (Figs 47–50) (*E. japonica*, *E. longicornis*, *E. tridens*) ... 15
14. Penis valves as in Figs 51 and 56; tegulae and hind tibiae mostly black; claws mostly with a conspicuous tooth . . . *E. alector*
- Penis valve as in Fig. 58; tegulae and hind tibiae partly white; claws mostly simple or with an inconspicuous tooth *E. minuta*
15. TL max exceeds TL min about 1.45–1.55 times (Fig. 3); the area between frontal crests mostly does not exceed the level of crests in dorsal view (Fig. 4); length of the flagellum 3.2–3.8 times of the head breadth *E. japonica*
- TL max exceeds TL min about 1.2–1.35 times (Fig. 7); the area between frontal crests mostly exceeds the level of crests in dorsal view (Fig. 8) 16
16. Valvura to valviceps length ratio (Fig. 9) mostly 0.45–0.56, baso-dorsal tip of the valviceps mostly angular (Morphbank images id=579799; Fig. 48); flagellomere 3 mostly longer than 0.7 mm; flagellum length exceeds 3 times of the head breadth; abdominal terga mostly with 3 pairs of large and 1 pair of small whitish flecks *E. longicornis*
- Valvura to valviceps length ratio (Fig. 9) mostly 0.52–0.63, baso-dorsal tip of the valviceps round (Figs 49–50, 57); flagellomere 3 mostly shorter than 0.7 mm; length of the flagellum mostly 2.4–3.1 times of the head breadth; abdominal terga mostly with 4 pairs of large and 1 pair of small whitish flecks *E. tridens*
17. Dorso-apical tooth of valviceps large, apical part of valvular duct goes clearly further from dorsal rim of valvura (Fig. 55) . . . *E. loktini*
- Dorso-apical tooth of valviceps small, apical part of valvular duct reaches almost the dorsal rim of valvura or going only slightly further from it (*E. basalis*, *E. tridens*) ... 18
18. Basal lobe of the valviceps short (Figs 49–50), length of the flagellum mostly 2.4–3.1 times of the head breadth . . . *E. tridens*
- Basal lobe of the valviceps long (Fig. 52), length of the flagellum 2.1–2.7 times of the head breadth *E. basalis*

Discussion

Phylogenetic analyses of the mitochondrial and nuclear sequence data strongly support monophyly of the group, suggesting that structural similarities of the males' penis valves between the species are not due to convergence but resulted by shared ancestry.

Species discrimination in *longicornis*-group. While most *Empria* species are externally rather homogenous, their differences in genitalia can be significant even between closely related species. Because of their external similarity, the structure of ovipositors and penis valves should be studied to identify the species reliably (e.g. Lindqvist 1968; Smith 1979; Zhelochovtsev & Zinovjev 1988; this study). It is generally true also for the *longicornis*-group species which are very closely related and resembling each other.

For an example as to why relying only on external morphology for species identification can be misleading, we consider the length of head behind the compound eye relative to the length (“breadth” in Benson 1938) of the eye. Benson (1938) regarded this as a good diagnostic character to distinguish *E. alpina* from other *Empria* species; however, albeit rarely, other species of the *longicornis*-group may possess the same character state as well (compare Figs 59 and 60). The most extreme in this regard are two females from northern England (see Morphbank images id=568477–568483) and Scotland, which have uniquely shaped head (strongly swollen behind the compound eyes) among *Empria* species, but the valvular structure is indistinguishable from *E. tridens*. ITS1 and 2 sequences from one of the specimens confirm this identification, being identical with the sequences H08-04a and 05-22 from typical specimens of *E. tridens* (see Figs 16, 18). Because the structures of ovipositors and the penis valves are rather uniform within the species but mostly distinct between them, the characters are reliable for differentiation of the *longicornis*-group species.



FIGURES 59–60. Head of *E. basalis* in dorsal view: 59, specimen (08-32a) with normally sized compound eyes; 60, specimen (BU95-01a) with small eyes. Note the differences in length of head behind the eyes due to differences in size of the eyes.

Females of most *longicornis*-group species can be distinguished quite easily by their ovipositors. The ovipositors of *Empria japonica*, *E. longicornis*, and *E. tridens* are rather similar, but can still be distinguished by some details. Differences between their host plant species have probably also impacted the divergence of the ovipositor structure in the *longicornis*-group species. Because all known host plant species of the group (this is also true for most *Empria* species) belong to Rosoideae and Dryadoideae (Rosaceae), the yet unknown host plants of *E. japonica*, *E. loktini* and *E. minuta*, belong very likely also in these subfamilies. Furthermore, Dryadoideae can most probably be excluded, as *Dryas*, the only Palaearctic taxon of this subfamily (see Hultén & Fries 1986; Potter *et al.* 2007), has arcto-alpine distribution, but *E. minuta*, *E. loktini* and *E. japonica* are distributed in more temperate regions, thus obviously have different host plants than *E. alpina* (*Dryas octopetala*). Differences in the ovipositor structure of *E. minuta* and *E. loktini* suggest that their host plant species are probably different. Because almost all type specimens (3 ♀, 1 ♂) of *E. loktini* were collected from the leaves of *Filipendula kamtschatica* (see Ermolenko 1971), this might be the likely host plant of this species. Larvae of *E. minuta* probably feed on some other Rosoideae species. *Empria japonica* has sometimes been found from the habitats where *Rubus idaeus* ssp. *melanolasius* is abundant (A. Shinohara, personal communication) and its ovipositor structure closely resembles *E. longicornis* and *E. tridens* which both feed on *Rubus idaeus*. Thus, *Rubus idaeus* or some other closely related species might be considered as possible host plant of *E. japonica*.

Males are more difficult to identify using genitalia alone than females by their ovipositors, but combining some other external characters with those of genitalia (see the key) allows identification of most of the males with confidence. Perhaps the most difficult species pairs to distinguish are *E. longicornis*-*E. tridens*, the males of *E. basalis*-*E. tridens*, and the males of *E. alector*-*E. tridens*. While it might be difficult to distinguish the males of

E. tridens from *E. alector* and *E. basalis*, the females of those species can readily be separated by their ovipositor structure (compare Figs 42, 45 with Figs 43–44). Also, their host plants are different: *Rubus idaeus* in *E. tridens*, *Filipendula ulmaria* in *E. alector*, and *Geum rivale* in *E. basalis*. Although *Empria longicornis* and *E. tridens* share the same host plant and it is sometimes difficult to distinguish their females solely on a morphological basis, according to the ITS sequence variation, *E. longicornis* is clearly distinguishable from *E. tridens* as well as from other species of the group. Because most of the females of *E. tridens* and *E. longicornis* are morphologically rather well separated (see the key) and the identifications match with the ITS sequence data (Fig. 18) there is not much doubt about their specific status.

Regarding species circumscription, the ITS sequence variation agrees well with morphological results. Though most of the species can be delimited quite well on a morphological basis (e.g. Figs 12–14) and the ITS sequences (Fig. 18), *Empria tridens*, which is morphologically rather variable (especially the males, see Fig. 14), cannot be delimited by the sequence variation alone (Figs 16–18). Nonetheless, most of the *E. tridens* females are morphologically sufficiently uniform (Figs 12–13) and we did not find external or ovipositor characters to divide specimens of *E. tridens*. Consequently we treat *E. tridens* as single species. Still, a method of agglomerative clustering (unweighted centroid method) of the analyzed set of specimens (females and males together) based on the Manhattan distance matrix calculated using a subset of binary coded parsimony informative DNA characters (ITS nucleotide sequences) with the length of the flagellomere 1, grouped the conspecifics together in accordance with their genitalia structure (Fig. 61).

Hypotheses on evolution of host plant use. For the LTAB clade (“*Pantridens*”: *E. longicornis*, *E. tridens*, *E. alector*, *E. basalis*) of ITS phylogeny (Figs 16–18) we consider *Empria tridens* as an “ancestral species”. *Empria tridens* is morphologically (Figs 12–14) and genetically (Figs 16–18) the most variable species with largest known distribution range in this group, while other species are less variable and partly overlapping (Figs 13–14) with *E. tridens*. Based on phylogenetic reconstructions of the ITS sequences (Figs 16–18) it is plausible that the ancestral host plant was *Rubus* and that *E. basalis* and *E. alector* diverged from *E. tridens* by switching to new host plants *Geum rivale* and *Filipendula ulmaria*, respectively. *Empria longicornis* on the other hand has diverged from *E. tridens* without switching host plant which also explains a remarkable similarity in their ovipositor structure. Ovipositors and penis valves of *Empria japonica* (host plant unknown) are quite similar to *E. tridens*, and if we accept the ITS phylogeny reconstructed using BALi-Phy (with indel characters included; Fig. 16) *Rubus* might have been an ancestral host plant for both, the LTAB clade and *E. japonica*. Sequencing of other nuclear DNA markers unlinked to rRNA operon is needed to test this.

Phylogenetic incongruence between nuclear and mitochondrial markers. One of the reasons why our mtDNA phylogeny is incongruent with morphology and nuclear DNA phylogeny might be frequent amplification of nuclear pseudogenes (numts) instead of actual mtDNA (e.g. Bensasson *et al.* 2001; Song *et al.* 2008; Viljakainen *et al.* 2010). Although we cannot completely rule out this possibility, it seems unlikely, because: 1) the full COI sequences neither contain any in-frame stop codons nor frame-shift causing indels; 2) different primers gave identical results; 3) typically high AT content (about 73%) for mitochondria of arthropods (Hassanin *et al.* 2005; Clare *et al.* 2008); 4) outside *E. longicornis* group (except *E. immersa* group), anomalous groupings of mtDNA sequences are absent (well definable groups and species are monophyletic, in preparation). However, we were unable to sequence good quality mtDNA from the specimen 06-06a (*E. tridens*), which might indicate amplification of numts in addition to mtDNA.

In case of rapid speciation, significant phylogenetic incongruence between different genes (i.e. incomplete lineage sorting) is expected from coalescent theory (e.g. Degnan & Rosenberg 2009). This could be one explanation for the incongruence between our mitochondrial and nuclear ITS phylogenies. Alternatively, the incongruence could be caused by mitochondrial introgression. If the hybridizations between closely related species are sufficiently rare, the identification of species based on nuclear DNA (and potentially morphology) will not be problematic, while the diversity of mitochondrial haplotypes among different species can be greatly affected (Chan & Levin 2005; Linnen & Farrell 2007), even to the point where mitochondrial diversity of one species is completely replaced by mitochondrion from another (e.g. Nevado *et al.* 2009). Because effective population size of mitochondrial DNA (mtDNA) is theoretically 4 times smaller (mtDNA is haploid and mostly uniparentally inherited) and evolves faster than nuclear DNA (Ballard & Whitlock 2004), mtDNA lineages in different species should become reciprocally monophyletic also faster. Since the opposite picture is seen in our nuclear and mitochondrial phylogenies (Figs 18, 20), it is possible that non-monophyly of mtDNA lineages of most of the species has been caused by

occasional hybridizations. However, additional nuclear markers and coalescent-based analyses are needed to distinguish between incomplete lineage sorting and mitochondrial introgression more reliably (e.g. Linnen & Farrell 2007).

Interestingly, there seems to be mitonuclear discordance also inside *E. immersa* group (cf. Figs 18 and 19–20), which have highly similar penis valves between species (like in *E. longicornis* group).

Potential barcoding markers for species. COI has been suggested as a universal barcoding marker for animal species identification (Hebert *et al.* 2003a; Hebert *et al.* 2003b). Because mtDNA cannot be used to identify species in the *Empria longicornis* group, some nuclear marker should be used instead of COI for barcoding. However, COI can be a suitable marker for other *Empria* species (unpublished observations). For all or most species of *Empria longicornis* group (and possibly for *immersa*-group as well), ITS1 alone (but not ITS2) has species specific sequence variability. Although *E. tridens* does not form a monophyletic group, it does not seem to share haplotypes with any other species (it is possible that some other nuclear marker(s) unlinked to rRNA operon might support the monophyly of *E. tridens*). Unfortunately, ITS1 is quite long (about 1000 bp, including parts of 18S and 5.8S rRNA) and rich in GC content and repetitive regions, all of which hinder sequencing of this marker. Nevertheless, the 3' end of the ITS1 sequence (amplicon length would be about 350–400 bp), potentially contains information to identify all species in the group (Fig. 62). Primers could be designed to amplify this region, but there could also be other nuclear markers which are more informative and easier-to-sequence. Either way, additional efforts are necessary, if identification of the species in *E. longicornis* group based solely on short DNA sequences is needed.

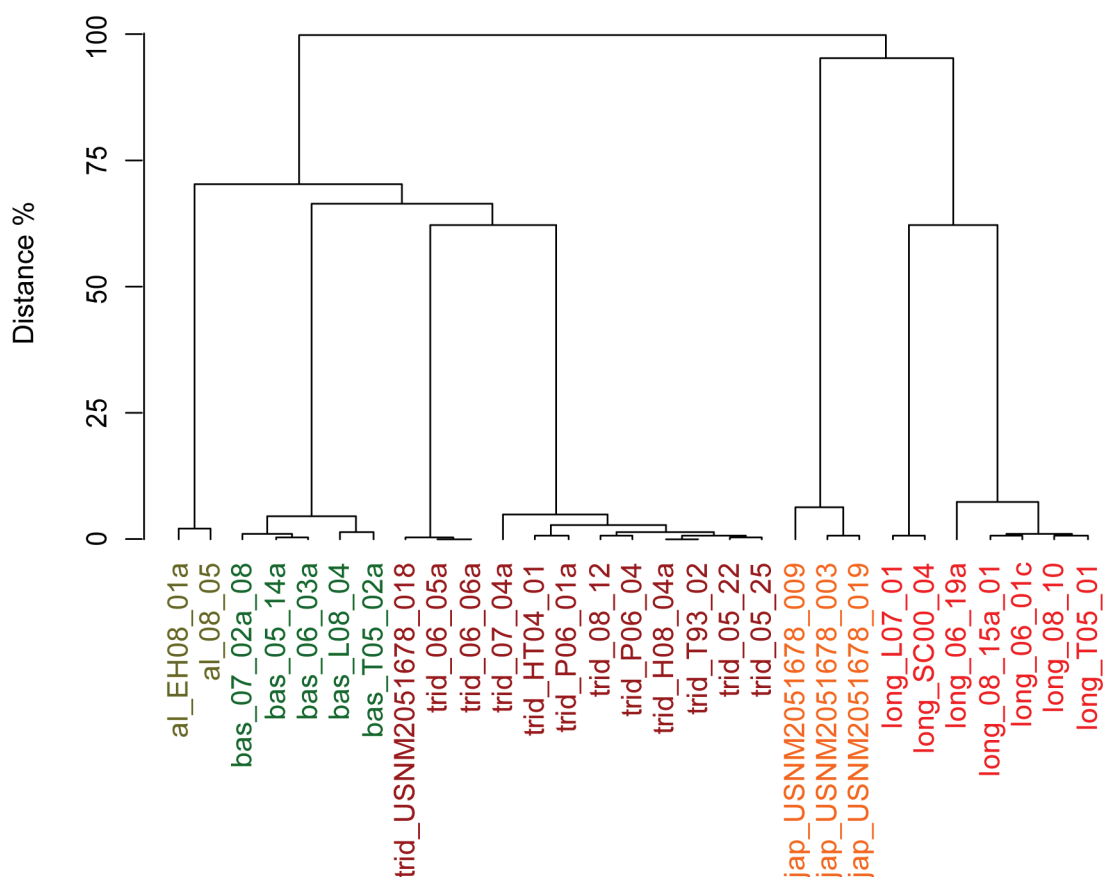


FIGURE 61. Dendrogram of similarity analysis of morphological (length of the flagellomere 1) and molecular characters (a subset of binary coded parsimony informative nucleotide positions of ITS sequences). Agglomerative clustering (unweighted centroids) of specimens' similarity data (Manhattan distance used). Specimens identified by genitalia structure.

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